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THE AMERICAN JOURNAL OF THE MEDICAL SCIENCES

SEPTEMBER, 1950

ORIGINAL ARTICLES

THE EFFECT OF POTASSIUM ON THE ELECTROCARDIOGRAM IN THE NORMAL DOG AND IN DOGS WITH MYOCARDIAL INFARCTION

By Samuel Bellet, M.D.

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Because of recent increase in the therapeutic use of potassium, it is desirable to re-evaluate the electrocardiographic alterations, particularly in the precordial leads that result from hyperpotassemia. In addition, the effect of potassium administration in the presence of myocardial damage as represented by various stages and degrees of myocardial infarction yields data of interest.

The effect of potassium on the electrocardiogram in the experimental animal has been studied by various authors[2,3,11,16,17]. The correlation of the electrocardiographic changes with the level of the potassium in the serum was first made by Winkler, Hoff and Smith[17] (1938). Many studies have been reported correlating the effects of varying grades of hypopotassemia and hyperpotassemia with the electrocardiogram in the human subject[4,5,10,14,15]. As far as we are aware, the only report relating to the effect of potassium in the presence of myocardial damage was made by Sharpey-Schafer[13] who studied the effect of oral potassium administration upon the inverted T wave in patients with hypertension and in patients with myocardial infarction. He gave no correlation of the ECG with serum levels of potassium and no mention of altered sensitivity of the damaged heart.

Method of Study. The method of study used in these experiments is described in detail in another report[1]. Control tracings consisted of the usual 3 limb leads and 4 precordial leads. During the administration of potassium practically continuous tracings were taken by the Visocardiette. The precordial leads CR3 and CR4 were used for this purpose because these derivations gave the most characteristic evidence of potassium

effects. In addition, endocardial leads were taken from the left ventricular cavity in a normal dog and in a dog with acute myocardial infarction. A total of 31 experiments were performed; these were divided as follows: 9 in normal dogs, 12 in dogs with acute infarction, 6 in dogs with healed infarction, the reversibility of electrocardiographic effects in 2 dogs, effect of potassium on the simultaneous endocardial and pericardial electrocardiograms in the normal and in the acutely infarcted dog.

Results. In general, the electrocardiographic alterations following the administration of potassium may be divided into 4 stages: *a*, alterations in T wave and ST segment; *b*, alteration in the P wave; *c*, QRS changes; *d*, arrhythmias and terminal cardiac mechanisms.

The T Wave and ST Changes. The alterations in the T waves and ST segment are of particular importance because they occur during the initial stages of potassium administration. The patterns observed varied significantly in the normal and damaged hearts. Three patterns of T and ST changes were observed: (a) normal pattern (pattern observed in normal hearts); (b) intermediate pattern (observed in dogs presenting slight grades

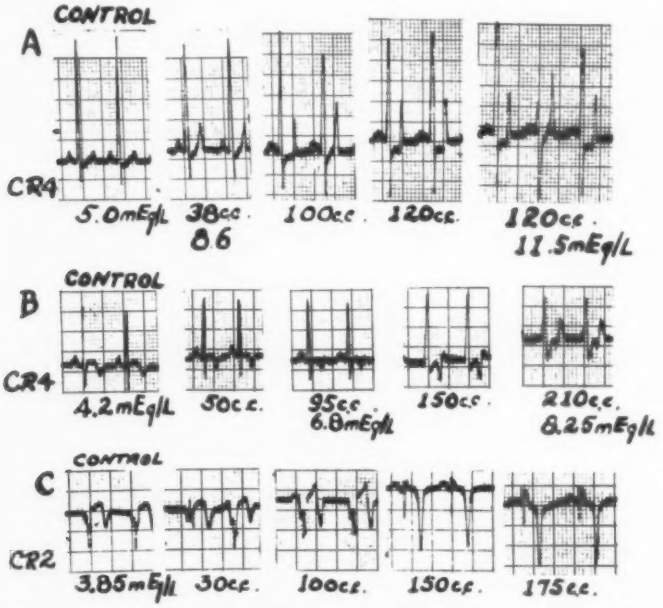

FIG. 1.—The 3 patterns of T waves and ST segment changes observed in normal and infarcted dogs.

(A) Normal dogs. Control CR4—Successive changes are recorded following increasing doses of isotonic potassium chloride. Below these figures are the potassium concentration in the serum in mEq. per liter. Note the increasing amplitude of the T wave with narrowing at the base and depression of the ST segment which tends to increase with increasing concentrations.

(B) Intermediate type. Control shows inverted T wave with slightly elevated RT segment. With increasing doses of potassium T wave inversion becomes more marked but amplitude of T wave tends to decrease. After 150 cc. RT segment becomes depressed and P wave disappears.

(C) Acute infarction pattern. Control CR2 taken 48 hours after coronary ligation. Note absent R waves and elevation of RT segment followed by inverted T wave. With increasing doses of potassium inversion of T wave becomes more marked, RT elevation disappears after 150 cc. Note increasing amplitude of inverted T wave with narrowing at the base. Configuration of final T wave change is not just similar to, but is a mirror image of, that observed in the normal. In this and following figures a few faint lines have been strengthened.

of myocardial infarction with subendocardial involvement) and (c) infarction pattern observed in the presence of a transmural infarction of the left ventricle (Fig. 1).

(a). *Normal Pattern* (Figure 1A). The patterns of T and ST changes in the normal dog are similar to that described by Winkler, Hoff and Smith[17] in their animals. However, these changes are more clearly observed in the precordial leads CR3 and CR4. The earliest change consisted of an increase in amplitude of the T wave. When markedly increased it was usually accompanied by depression of the ST segment (Fig. 1A). This pattern was observed in 9 normal dogs. In 2 of these, the T waves increased only slightly in amplitude. In the remainder, the amplitude of the T waves gradually increased until it reached almost the amplitude

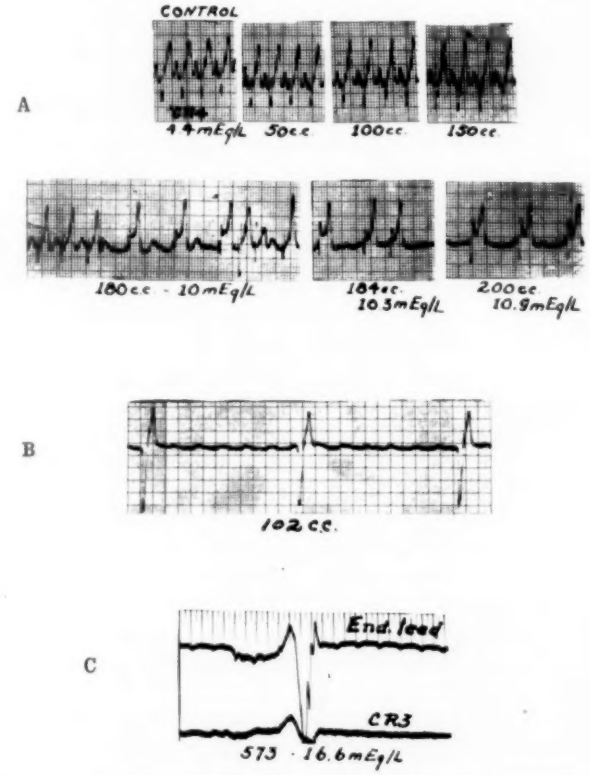

FIG. 2.—(*A*) Effect of potassium in the presence of a healed myocardial infarct (Grade 3) 30 days after ligation of the anterior descending branch of the left coronary artery. Control CR3—note absent R waves followed by upright T waves. Following administration of potassium note increasing amplitude of T wave with narrowing at the base similar to that observed in normal dogs. After 180 cc. note elevation of RT segments and absent P waves. These findings are more marked after 184 to 200 cc. Note the development of a late R wave. (*B*) *Healed infarct.* (Grade 3) 37 days after coronary artery ligation. After 102 cc. of potassium note the slow idioventricular rhythm and presence of auricular tachycardia with a rate of 150 per minute.

(*C*) *Terminal cardiac mechanism in a normal dog.* The upper strip is the endocardial lead taken with the electrode tip in the left ventricle. The lower strip is the normal CR3. Note the presence of P waves recorded with the endocardial lead and absence in the precordial lead CR3.

of the R wave. The QT interval tended to be shortened. In 6 dogs with healed anterior infarctions, T wave changes were similar to those observed in the normal, even though an electrical window was present. However, the ST segments became elevated rather than depressed as in the normal (Fig. 2A).

(b). *Intermediate Type* (Figure 1B). This pattern consisted of a gradual diminution in the amplitude of the already inverted T wave, rather than an increase. The amplitude of the T wave remained relatively small. This pattern was observed chiefly in those animals with slight to moderate grades of myocardial infarction with subendocardial involvement (Grade 1 and 2 infarcts), but was not observed in the transmural infarcts.

(c). *Acute Infarction Pattern* (Figure 1C). These animals in the control electrocardiogram showed the typical pattern of acute transmural infarction; namely, absence of the initial upward deflection, inverted cove-shaped T waves and some ST segment elevation. As potassium was administered the inversion of the T wave tended to increase, the ST segment elevation became less marked and the final T wave changes developed which were similar to, but exactly opposite in direction from, the high amplitude peaked T wave observed in the normal. These changes are similar to those reported in the human by Sharpey-Schafer in healed infarcts[13]. In 3 dogs of this group the amplitude and configuration of the T wave remained unaltered during potassium administration until the stage of bundle branch block.

Alterations in the P Waves. Coincident with the T wave and ST changes, the P waves increased in amplitude and became peaked. They then gradually decreased in size and became broadened. The auricular rate gradually decreased with the development of sino-auricular or intra-auricular block. This decrease in auricular rate usually occurred coincidentally with the occurrence of intraventricular block. The auricular rate became slower and the auricular complexes usually disappeared with the development of more marked grade of QRS widening (Fig. 1B). In 3 instances auricular activity was observed in association with widening of the QRS complexes and complete auriculo-ventricular heart block. In one, where death was due to cardiac standstill, auricular activity was observed, even though ventricular activity had ceased (Fig. 2, B and C). This point is of interest because the auricular activity was observed in the endocardial leads taken from the left ventricle and was not clearly shown by the precordial leads. In the 2 other experiments on healed infarcts, auricular activity was observed (rate 150 per min.) during a slow idioventricular rhythm with marked widening of the QRS complexes. In these 2 instances, even with a relatively slow ventricular rate, the P waves were inverted, suggesting a pacemaker in or close to the A-V node. Similar evidence of auricular activity at the height of potassium effect was observed by Nahum and Hoff[11] and Wiggers[16].

Alterations in the P-R Interval. The P-R interval was not significantly altered in the early stages of potassium administration. However, at the time of ST segment depression slight prolongation of the P-R interval was noted. The P wave became broadened to occupy almost all of the prolonged P-R interval in many instances. In 2 dogs where auricular activity was noted with a slow idioventricular rhythm, partial A-V heart block, which gradually changed into complete A-V dissociation, was observed.

Alterations in the QRS Complex. The development of intraventricular block

was considered to be an evidence of serious toxic effect. It occurred at a mean serum concentration of potassium of 11.3 mEq. in the normal and at an earlier stage in the infarcted animals[1]. The initial widening of the QRS complex gradually progressed with continuance of potassium administration until marked widening up to 0.24 to 0.28 sec. occurred immediately preceding the death of the animal. When bundle branch block occurred, as far as we could determine, the right branch was the one involved. In dogs with transmural infarction involving the anterior wall of the left ventricle, right bundle branch block was observed as in the normals. However, its configuration was altered because of the infarction. As potassium was given, a late R wave appeared which gradually increased in amplitude until it attained a height of 8 to 11 mm. (Fig. 3 B, lower strip).

Effect on Ventricular Rate. The heart rate in the controls depended on the type of anesthesia. Under morphine sulphate the control rates were slow (45 to 60 per min.); under nembutal the control rates were more rapid (150 to 180 per min.). In the former group

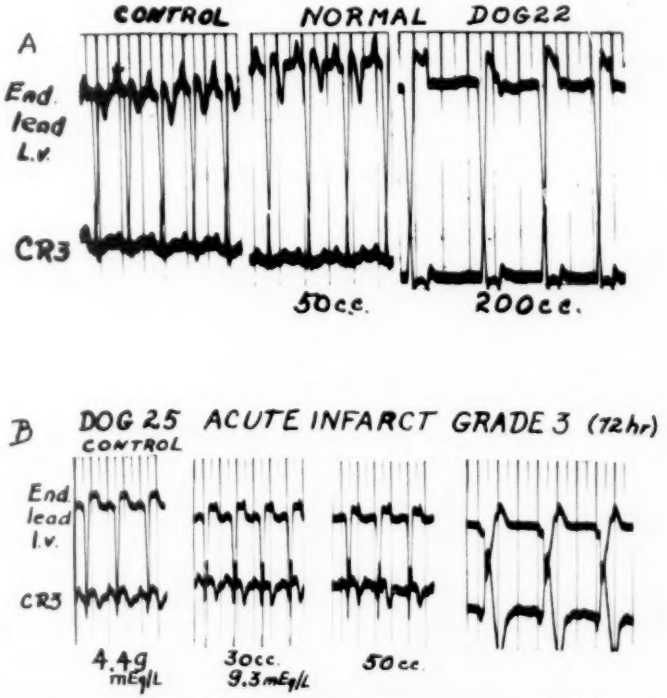

FIG. 3.—*(A)* Simultaneous record of endocardial lead with the tip in the left ventricle and CR3 in a normal dog. The first record represents the control tracing. Note the absence of the R waves in the endocardial lead followed by the inverted T wave. After 50 cc. note increasing inversion of T wave in the endocardial lead with slight elevation of the RT segment. After 200 cc. note elevation of the RT segment and endocardial lead and inversion of T wave and depressed ST segment in the precordial lead.

(B) Acute infarct. Simultaneous records of endocardial lead from left ventricle and CR3. Control shows absent R waves and elevation of RT segment. In endocardial lead absent R wave followed by T wave in CR3. Note increasing degree of T wave inversion in CR3 with practically no change in the endocardial lead. The last strip shows the presence of a marked degree of intraventricular widening with the appearance of a late R wave.

potassium administration led to a slight increase in heart rate, which reached its maximum at the height of the T wave changes. This was probably the result of a sympatheticomimetic effect. In dogs with nembutal anesthesia there was a tendency toward slowing of the ventricular rate as the T wave changes occurred. The ventricular rate gradually decreased thereafter in all animals with the development of intraventricular block and as the degree of intraventricular block increased and slow idioventricular rhythm (20 to 30 per min.) developed.

Arrhythmias. A normal sinus rhythm was usually observed in the control tracing. Following the development of intraventricular block the auricular rate slowed markedly in a short time due to the development of intraventricular block. In 3 dogs we noted the occurrence of periods of partial A-V heart block (2 to 1) prior to the development of a complete A-V heart block with an auricular tachycardia (200 per min.). At various periods after development of intraventricular block runs of rapid ectopic rhythm, associated with aberrant ventricular complexes, appeared with a rate ranging from 140 to 220 per minute. This was probably a nodal or ventricular tachycardia (Fig. 4, Strip 4). These episodes lasted for brief periods of time, usually disappeared even with a continuance of potassium administration, and gave way to widened idioventricular complexes with a slow rate (about 20 per min.). When ventricular standstill occurred as a terminal mechanism, it was usually preceded by a slow idioventricular rhythm with markedly widened QRS complexes. When ventricular fibrillation was the terminal mechanism, it was usually preceded by a ventricular tachycardia of a relatively slow rate characterized by marked widening of the QRS complexes.

Endocardial Potentials. Simultaneous records of the endocardial potentials with the electrode placed on the left ventricula were taken with precordial leads (CR4) in the normal dog. These showed a T wave configuration which was exactly an inverted image of the lead on the endocardial side. As potassium was administered the amplitude of the T wave increased in the precordial lead and became more inverted in the endocardial lead. When the ST segment deviations occurred they were opposite in direction in these 2 leads (Fig. 3A). In the presence of transmural infarction both endocardial and precordial leads (CR3) showed absence of the R wave (Fig. 3B). A definite RT segment elevation was observed in the endocardial lead but was slight in the precordial lead. This is probably due to the fact that the endocardial lead gives a more sensitive recording of a small area with the electrode in direct contact with the injured portion, whereas the epicardial lead records the potentials of a larger portion of the epicardium (Fig. 3B).

Reversibility of Potassium Effects on the Electrocardiogram. Two experiments were devised to determine the reversibility of potassium effects after its administration was stopped after various toxic levels were reached. On 2 occasions in the same experiment we were able to give this electrolyte to the stage of bundle branch block and ventricular tachycardia (rate 215 per min.) and the electrocardiogram returned to a normal configuration with a corresponding fall in the serum potassium. Once intraventricular block with a slow idioventricular rhythm occurred, we could not reverse the change and death followed with the development of slow ventricular fibrillation and a high level of the serum potassium (17.8 mEq. per liter) (Fig. 4).

Terminal Cardiac Mechanism. In all

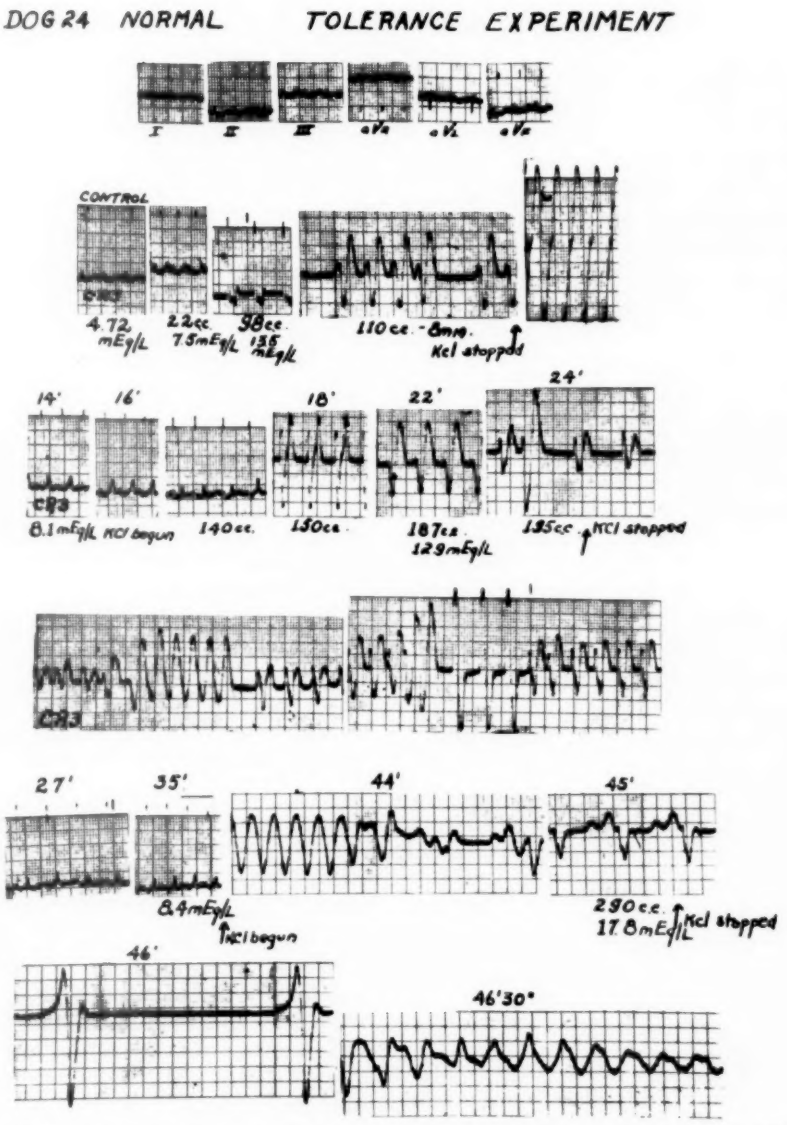

FIG. 4.—*Tolerance Experiment.* Note configuration of T wave in control tracing CR3 and the normal level of serum potassium. Note the typical changes of T wave and ST changes with increasing doses of potassium. After administration of 110 cc. in 8 minutes, potassium chloride administration was stopped with presence of ventricular or nodal tachycardia and intra-auricular block. At 14 minutes following beginning of potassium administration the normal cardiac mechanism has recurred, the T wave is somewhat greater in amplitude than the normal control and the serum potassium level is 8.1 mEq. per liter. Potassium administration was resumed 2 minutes later (16 minutes after beginning of experiment). Toxic changes are observed and at 24 minutes, when bundle branch block appeared with sinus bradycardia, potassium chloride administration was again stopped. Ventricular tachycardia again ensued and at 35 minutes the cardiac mechanism had again returned to normal. The T wave and the serum potassium concentration were almost similar to that observed at 14 minutes. Potassium administration was started again and stopped at 45 minutes after a total of 290 cc. had been administered and the serum level was 17.8 mEq. per liter. Note the occurrence of the slow idioventricular rhythm and ventricular fibrillation in one and a half and one-half minutes thereafter respectively.

the normal dogs the terminal mechanism at death was cardiac standstill. In 7 of the 9 normal dogs artificial respiration was used during the experiment. Of the infarcted dogs, one Grade 1 chronic infarct, one Grade 1 (acute) and two Grade 2 (acute) died in cardiac arrest. Two of the 4 had received artificial respiration during the experiment. All of the other dogs (15) with acute myocardial infarction (all with chronic infarcts) received artificial respiration and died with ventricular fibrillation. In the series of Winkler, Hoff and Smith[17] where potassium was administered in a manner similar to that used in our experiments, death occurred in ventricular standstill. The above data would indicate that the factor chiefly responsible for the mechanism of death was the presence of myocardial infarction. The combination of potassium and myocardial infarction usually led to ventricular fibrillation.

Discussion. *Electrocardiogram as an Indication of Hyperpotassemia.* The above data indicate that the electrocardiogram is a fairly good criterion of hyperpotassemia in the normal dog, and in the healed stage of infarction.

In the later stages of hyperpotassemia with development of intraventricular block, rapid ectopic rhythms and a slow type of idioventricular rhythm with marked degrees of QRS widening were observed in all of the dogs, both normal and those with infarcts. However, the duration of these toxic effects was relatively shorter in the dogs with severe grades of acute infarction because of the lower serum level at which death occurred. Although these patterns (consisting of intraventricular block, QRS widening and a slow idioventricular rhythm) are sometimes regarded as characteristic of hyperpotassemia, they are probably also caused by the action of other drugs, such as mercurials[9] and quinidine, and are also seen as a result of anoxia.

Physiologic Considerations. Potassium is important in the normal transmission of the cardiac impulse and for the normal activity of muscle and nerve. This cation normally plays an important role in polarization of all cell membranes and thereby profoundly affects the electrocardiogram. The endocardial potential is similar to but opposite in direction to the epicardial potential. It is for this reason that in the presence of a transmural infarction, the changes are opposite in direction to those in the normal because we are recording endocardial potential through an electrical window. In the presence of subendocardial infarction with varying degrees of intramural and epicardial infarction there is produced a combination of epicardial and endocardial effects which in varying degrees tend to neutralize one another, resulting in the intermediate pattern (Fig. 1B).

An excess of potassium in the serum apparently affects first the repolarization mechanism (T wave and ST segment) long before it attains the toxic range when the depolarization (QRS) is affected. Whether this is due entirely to alterations in potassium in the serum or to an altered relationship between extra and intracellular potassium is difficult to state. The available data suggest that the T and ST changes are the result chiefly of an increase in the extracellular potassium concentration because the T and ST changes often occur very soon after the beginning of the administration of potassium chloride, at which time a rapid initial rise in serum potassium[1] would appear to indicate that very little potassium has entered the cellular phase. It has been suggested that the lethal dose of potassium is attained when potassium reaches a certain critical level in the serum[3]. During the stage of acute in-

farction (and probably other types of acute heart damage) due to necrosis and death of cells there is a local liberation of potassium which bathes not only the injured cells but the borderline zone of relatively uninjured tissue. This profoundly affects their function and electrical potential. When potassium is added from without to the conditions that already exist a critical toxic level would be reached earlier than would occur in a relatively normal heart. This may at least partially explain the lower serum potassium concentration at death in severely injured hearts during acute infarcts and the higher concentrations observed at death in the normal and healed infarcts. Potassium concentrations in excess have been known to reduce the excitability of nerve and in still higher concentrations to block nerve conduction. It is probably by a similar mechanism that bundle branch block of increasing grade is produced.

We are unable to explain why conduction in the right bundle branch fails in many cases before the left. The development of intra-auricular block is probably due to a mechanism similar to that observed in the ventricles, and, because of the smaller muscle mass, it probably occurs at a somewhat lower concentration.

Reversibility of Potassium Effects in the Electrocardiogram. It is interesting that in the presence of normal renal function that the potassium effects are rather easily reversible even when severe toxic stages are attained (Fig. 4). There are apparently no cumulative effects of potassium as with digitalis and the maximum effects are observed immediately at the time of its administration. Rapidity and completeness of reversibility of effects have an important bearing on the clinical problem relative to potassium administration.

Relation of Experimental Observations to the Clinical Problem of Hyperpotassemia. It is difficult to relate all of these findings to the clinical problem; however many of them can be correlated with changes observed in the human. The initial effects of potassium administration and later toxic effects are similar to those observed in the human. The reversibility of electrocardiographic effects upon cessation of administration has been observed in the human, even in the toxic range. The experimental data suggest that the use of potassium to differentiate between the inverted T wave of myocardial infarction and other conditions, as suggested by Sharpey-Schafer[13], is of limited value in the human subject; its use is probably not without some added risk. Hyperpotassemia in the human subject is usually complicated by the presence of an altered metabolic and electrolyte state which includes alterations in phosphates, sodium, calcium and magnesium. These add to the complexity of the clinical picture and electrocardiographic pattern.

Summary. 1. The effects of potassium adminstration on the electrocardiogram in 31 dogs are reported. Of this group there were 9 normal dogs, 12 with acute infarcts, 6 with healed infarcts, and the endocardial potentials of the left ventricle were determined in one normal animal and in one with an acute infarction; the determination of the reversibility of electrocardiographic effects after toxic doses was determined in 2 dogs.

2. Three patterns of T and ST changes were observed: *a*, normal, consisting of increase in amplitude of T wave with narrowing of the base and depression of the ST segment; *b*, intermediate type, observed in hearts with small infarcts which involved chiefly the endocardial portion of the left ventricle, consisting of diminution in ampli-

tude of the T wave and inversion of the T wave. The amplitude of the T wave remained small; *c*, acute infarction pattern, observed in transmural infarcts and characterized by an increase in amplitude of the inverted T wave, its configuration practically an inverted image of that observed in the normal. Subsequent toxic effects which consisted of bundle branch block, ventricular tachycardia, and bradycardia were observed in both normal and infarcted dogs.

3. The terminal cardiac mechanism in normal dogs was cardiac arrest. In those with myocardial infarcts, death in most instances was due to ventricular fibrillation.

4. There was no evidence of cumulation of potassium effects. Maximum electrocardiographic changes were observed at the time of its administration. Even at the stage of marked QRS widening and ventricular tachycardia, the electrocardiographic effects were reversible upon cessation of potassium administration.

5. The relationships of the experimental data to the clinical problem of hyperpotassemia in normal and infarcted hearts—similarity of initial and toxic effects, and of reversibility, complications and so on—are discussed.

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THE EFFECT OF DIFFERENT GRADES OF MYOCARDIAL INFARCTION UPON THE TOLERANCE TO POTASSIUM: AN EXPERIMENTAL STUDY IN DOGS

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The recent advances in potassium therapy in patients with hypopotassemia and other disease states[10,13,14] have emphasized the need for a safe method for the administration of this ion. The problem of determining the therapeutic range and early toxic action is of considerable importance. Chemical estimation, while highly desirable, is not always available initially or during the various phases of potassium administration. There is little evidence at hand relative to the variation of the toxic effect of potassium in the presence of myocardial damage, as compared to the normal. Recently, Elkinton and Tarail[17] commented: "The limited experience with potassium therapy in patients with organic cardiac disease and associated potassium deficit permits no comment concerning possible cardiac contraindications." In view of the well known toxic effects of this electrolyte[7,11,12,15,16] it seemed to us advisable to accumulate additional data relative to the early toxic effects, margin of safety, and the extent to which these factors are modified by myocardial damage.

As potassium produces its toxic effect chiefly on the heart, and as this electrolyte is generally administered to patients with varying degrees of myocardial damage (factors which could conceivably alter the toxic dose) we have studied the dog further to elucidate this point.

Winkler, Hoff and Smith[20] first correlated the electrocardiographic changes which followed the administration of potassium with the serum concentration of this cation in the normal dog. They found that alterations in the T wave appeared at concentrations of 5 to 7 mM. per liter, depression of the ST segments at 8 to 10 mM. per liter, beginning intraventricular block at concentrations of 10 mM. per liter, disappearance of the P waves at 9 to 11 mM. per liter, and cardiac arrest at 14 to 16 mM. per liter. Since only a few samples were taken by these authors, most of these values were obtained by interpolation, using the assumption that the concentration of potassium in the serum increased linearly with time. These findings were similar to those later observed by other investigators[2,3].

Material and Method. A total of 28 dogs weighing from 10 to 19 kg. was used for these experiments. The following types of experiments were performed:

TABLE 1.—NUMBER AND TYPES OF EXPERIMENT

	Number
Normal dog	8
Acute Infarction (24 to 48 hours after coronary artery ligation)	12
Healed stage of myocardial infarction (30 to 36 days after coronary ligation)	6
Determination of reversibility of effects	2
Total	28

Potassium was given intravenously to these animals (10 cc. of an isotonic solution, 1.14% or 155 mEq. per liter per minute) until death ensued. Practically continuous electrocardiographic tracings were taken with a Sanborn Visocardiette during this period. Control electrocardiograms consisted of Leads 1, 2, and 3 and the precordial Leads CR1, 2, 3, 4, and 5. During the recording of continuous tracings, Leads CR3 or CR4 were used because they show the most marked changes. A control sample of blood was taken from the femoral artery before potassium was given and additional samples were taken thereafter, simultaneously with significant alterations in the electrocardiogram. A final sample was taken immediately upon death of the animal. In all, from 4 to 8 blood samples were collected during each experiment. These samples of arterial blood were collected in oiled syringes for electrolyte determination. The concentration of potassium was determined by the Beckman flame photometer. For each specimen, a minimum of 3 readings was made, each reading being accompanied by a reading of the standard closest to that of the sample. Duplicate estimations were performed. The standards as prepared contained sodium and trichloroacetic acid in concentrations closely approximating that in the deproteinized specimen. Recoveries of potassium added to serum varied from 98 to 100%.

A continuous record of the blood pressure was made by introducing a cannula in the femoral or carotid artery and the changes in blood pressure were correlated with the electrocardiogram and the level of the serum potassium (Fig. 3).

The following types of light anesthesia were used in determining response to potassium: morphine sulphate, 10 mg. per kg. in 5 dogs, and intravenous sodium pentobarbital in the remainder. Artificial respiration by tracheal cannula was used in all dogs under sodium pentobarbital anesthesia, but not in the morphinized dogs.

Dogs in which myocardial infarction was produced were prepared in the following manner: the animals were anesthetized by the administration of Nembutal or ether. Artificial respiration was instituted. The chest was opened and the heart exposed by opening the pericardium. The anterior descending branch of the left coronary artery and vein were doubly ligated close to their points of origin in the first 5 dogs. In the remaining 14 animals the anterior descending coronary was dissected free at a point about 1 cm. from the origin and the artery alone ligated. This operation usually produced larger infarctions.

The response of the animals to potassium was tested at varying periods after operation as indicated in Table 1. Following death of the animal from potassium intoxication, the heart was carefully examined, the extent of the infarct noted and classified into 1 of 4 categories (Fig. 1). A Grade 1 infarct involved only a small portion of the apex together with the subendocardial portion of the heart muscle. A Grade 2 infarct involved a somewhat larger area. A Grade 4 infarct was the largest infarct encountered and involved most of the anterior portion of the left ventricle extending to the lateral wall. A Grade 3 infarct was intermediate between Grades 2 to 4.

In 2 experiments the reversibility of toxic effects was determined by administering potassium until the following electrocardiographic effects were noted: tall T waves, initial bundle branch block, and high grade intraventricular conduction defect. When these effects were observed, potassium administration was stopped until the ECG returned to normal[1]. The injection was then resumed until a further stage of toxicity, and the administration again stopped until an irreversible stage appeared.

Results. *The Effect of Acute Myocardial Infarction on the Serum Level at Death.* Normal dogs died with serum potassium levels which ranged from 13.9 to 18.0 mEq. per liter (average 16.5 mEq. per liter) (Table 2 and Fig. 2). Dogs with Grade 1 infarcts reacted similarly to normal dogs and died with serum potassium levels between 17.1 and 18.2 mEq. per liter (average 17.7 mEq.). Dogs with Grade 3 and Grade 4 infarcts exhibited a definite diminution in potassium tolerance. Dogs with Grade 3 infarcts died with serum potassium levels ranging from 10.5 to 16.3

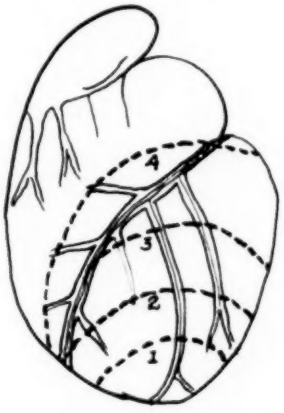

FIG. 1.—This diagram illustrates the approximate areas involved in the various grades of infarction. A Grade 1 infarct involved the anterior apical portion of the left ventricle and sometimes the subendocardial portion of the heart as well. A Grade 2 involved a still larger area, and a Grade 3 extended over most of the anterior portion of the anterior wall of the left ventricle. A Grade 4 was the largest infarct observed and involved practically the entire portion of the left ventricle extending from the anterior to the lateral wall and occasionally involving the right ventricle near the interventricular septum.

TABLE 2.—CORRELATION OF SIGNIFICANT ECG CHANGES WITH SERUM CONCENTRATION OF POTASSIUM

Normal	*Dog No.*	*Control (mEq./l)*	*Initial BBB (mEq./l)*	*Vent. Tachy. (mEq./l)*	*Serum Conc. at death (mEq./l)*
	21	4.1	10.9	12.7	16.1
	13	3.13	10.1	11.6	13.9
	12	5.0	11.5	15.8	18
	3	4.75		11.4	16.2
	20	4.87	13.6	15.1	18.3
	22	4.46	10.2	13.0	16.6
	Mean	4.4	11.3	13.2	16.5
ACUTE					
Grade 1 Infarction 24 to 48 hours.	4	4.4	8.9		17.1
	8	4.2	8.25	12.9	18.2
	Mean	4.3	8.6		17.7
Grade 2	11	5.0			12.7
Grade 3	10	4.0	8.54	12.6	13.1
	1	4.25	6.40		16.6
	2	3.85	6.80	10.25	10.5
	Mean	4.03	7.2	11.4	13.4
Grade 4	7	5.0		8.9	9.1
	9	3.72	7.0		9.0
	17	5.03		10.4	10.4
	Mean	4.58	7.0	9.7	9.5
CHRONIC					
Grade 1	18	4.87	10.9	11.9	18.0
Grade 2	23	4.41	10.9		19.9
Grade 3	14	4.46	10.9	11.4	13.1
	26	5.15	10.0	13.1	15.3
	27	4.18	11.2	14.3	15.1
	19	3.44	11.4	13.0	14.4
	Mean (Grade 3)	4.3	10.8	12.9	14.4

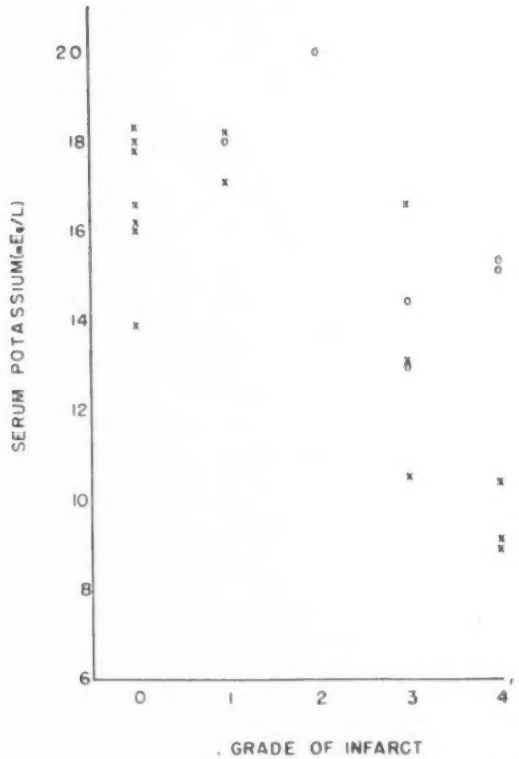

FIG. 2.—The serum potassium concentration at death is plotted against the grade of infarction. X marks dogs with acute infarctions; 0 dogs with healed infarctions. It will be observed that the serum level of potassium at death is significantly lower in the acute Grade 3 and 4 infarcts than in the normal control animals.

mEq. per liter (average 13.4); Grade 4 dogs died between 9.1 to 10.4 (average 9.5). It is evident from Table 2 and Fig. 2 that dogs with moderate and severe grades of acute myocardial infarction died at lower serum concentrations of potassium than the normal controls. In general, the serum level at death was lower in the animals with the largest infarcts. The statistical correlation between the grade of infarction and the serum concentration at death was -0.80 ($p = < .001$).

The Serum Level at which Intraventricular Block Appeared. More striking than the comparison of the lethal serum concentrations of potassium in the normal animals and those with moderate and large infarcts is the comparison of the serum concentration with progressive electrocardiographic effects in the normal dog and in dogs with myocardial infarction. When potassium is given to a normal dog up to the point of death, 4 chief changes are observed in the electrocardiogram with progression of toxic effects. These consist of: *a*, T wave and ST segment changes; *b*, initial intraventricular block which progresses to marked degrees of widening; *c*, ventricular tachycardia; *d*, death by ventricular fibrillation* or ventricular

* Wiggers[18] has pointed out that the fibrillation resulting from potassium administration is slower and coarser than that resulting from faradic stimulation. In potassium fibrillation, the waves are more regular in course, frequency and velocity.

TABLE 3.—THE CORRELATION OF THE ECG CHANGES WITH THE SERUM POTASSIUM LEVELS IN A NORMAL DOG, AN ACUTELY INFARCTED DOG, AND A CHRONICALLY INFARCTED DOG.

Exp. No.	*Weight of Dog (kg.)*	*Amt. of KCl (1.14%) injected (c.c.)*	*Duration of Injection (min.)*	*Rate of Injection (cc./kg./1 min.)*	*Serum Samples taken after c.c. of KCl*	*Serum K (mEq./l)*	*EKG Changes*	*Age of Infarct*
				Normal Dog				
20	19	356	36.0	0.52	0	4.87	Normal	None
					26	7.90	Initial T wave	
					87	10.7	ST segment depressed	
					180	12.2	S–A Block	
					264	13.6	I.V. Block	
					306	15.1	Vent. tachy.	
					346	18.0	Slow idiovent. rhythm	
					356	18.3	Vent. standstill	
				Acute Grade 4 Infarct				
9	14.2	171	20.2	0.60	0	3.72	Normal	72 hour
					124	7.00	S–A Block	
					130	7.40	Slow rate	
					150	7.70	I.V. Block	
					162	8.50	Slow idiovent. rhythm	
					171	9.00	Vent. fib.	
				Chronic Grade 3 Infarct				
19	15.0	283	28.9	0.65	0	3.44	Normal	32 days
					50	7.74	Initial T change	
					116	9.90	S–T depression	
					150	10.7	S–A Block	
					228	11.4	Early I.V. Block	
					245	13.0	Wide I.V. Block	
					265	13.0	Vent. tachy.	
					283	14.4	Vent. fib.	

standstill. Table 3 shows the correlation of the electrocardiogram with the serum level of potassium in 3 representative experiments: in normal dogs with acute infarction, and in dogs with healed myocardial infarction. While T wave and ST segment changes are evidence of potassium effect which occurs with increasing concentration of potassium, we consider the occurrence of intraventricular block as a definite toxic effect and progessive electrocardiographic changes as evidence of increasing toxicity. From Table 2 it will be seen that the initial and progressive electrocardiographic effects develop at lower serum levels of potassium in dogs with acute infarctions. For example, the mean serum concentration at which intraventricular block appeared initially was 11.3 mEq. per liter for the normal dogs, 8.6 for dogs with Grade 1 infarcts, 7.2 for Grade 3 infarcts, and 7.0 for Grade 4 infarcts; ventricular tachycardia also developed at a lower concentration in the infarcted dogs, but this arrhythmia was difficult to correlate with serum levels because it was not present in all dogs and was transient in character.

We were unable to find any significant correlation between the dose of potassium chloride administered per kg. of body weight and the serum potassium level at death. This confirms previous work in dogs[20] and in cats[3]. As has been reported, the cardiac changes appear to be correlated with

the level of potassium in the serum, rather than with the total dose of this cation given.

The Effect of Healed Myocardial Infarction on the Tolerance to Potassium. Six dogs were given intravenous potassium in the manner described during the healed stage of myocardial infarction, 1 month or more following ligation of the anterior descending branch of the left coronary artery. From Table 2 it can be seen that included in this group were 4 animals with Grade 3 infarction, one with Grade 2 and one with Grade 1 infarcts. These chronic infarctions generally tended to be smaller in size than the acute infarctions because of the healing that had occurred. None of these dogs appeared clinically to be embarrassed by the infarction, nor was any animal in cardiac decompensation.

In dogs with Grade 1 and 2 healed infarctions the concentration of serum potassium at which significant electrocardiographic alterations and death occurred did not differ from the values obtained in the normal control animals. However, the mean values for the serum potassium at death in dogs with Grade 3 infarcts tended to be lower by about 2 mEq. per liter. This difference was not found statistically significant.

The Reversibility of Potassium Effects. It is important to know how far the electrocardiographic evidence of potassium toxicity can develop and still permit return to normal upon cessation of the potassium administration. In 2 experiments, the dog was able to make a complete recovery after the appearance in the electrocardiogram of: *a*, T wave and ST segment changes; *b*, initial bundle branch block and progressive intraventricular block; and *c*, rapid ventricular tachycardia. After a slow idioventricular rhythm with marked widening of the QRS complexes had occurred the process was not reversible. This observation may have some bearing on the clinical administration of potassium.

Relation of Potassium Administration to Increase in Serum Concentration. In 4 experiments Winkler, Hoff and Smith[20] correlated the electrocardiographic changes in hyperpotassemia with the serum concentration of this cation. This work was done only in normal dogs and the correlation of these values was obtained by interpolation. These investigators plotted the serum potassium levels against time and were able to connect their 3 or 4 points by a straight line. Thus they assumed that the rise in serum potassium concentration was a linear function of time. This enabled them, by interpolation, to arrive at the serum concentration at any time during the administration of potassium chloride. Since the rise in serum potassium would depend upon several factors, such as the rate of urinary excretion and the percentage of administered potassium that moves intracellularly, it seems unlikely that the rise in serum potassium would be a simple linear function. Fig. 3 illustrates the rise in serum potassium plotted against time in our experiments in 5 normal dogs. These curves are not linear and tend to show an initial rise in potassium concentration, then a tendency to plateau, followed by a terminal rise. All potassium values shown in the Figure were obtained by chemical estimation.

The serum concentration which follows a single injection of potassium does not behave in a simple manner. Within 15 to 25 minutes after the injection of radioactive potassium the serum concentration falls to a minimum value, a large portion of it entering the liver cells, and this is followed by a secondary rise reaching a maximum value 40 minutes after injection[6,9,19].

Blood Pressure Changes. Correlation of the blood pressure with the various

electrocardiographic alterations during the administration of potassium revealed the following: during the T wave and ST segment alterations and during the initial stage of intraventricular block, the blood pressure did not vary significantly from the control level; the blood pressure tended to decrease slightly during the more marked grades of bundle branch block; with the onset of ventricular tachycardia, there was usually observed a sudden fall in blood pressure to about one-half the control value; the pressure rose with the end-

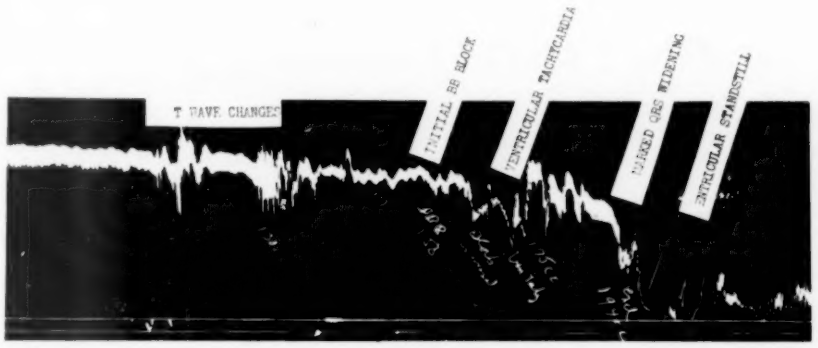

FIG. 3.—The continuous blood pressure records in a normal dog during the administration of potassium. It will be noted that the decrease in blood pressure was relatively slight during T wave changes and showed a definite downward dip coincident with the development of ventricular tachycardia. Thereafter the blood pressure tended to decrease gradually with the development of marked degrees of QRS widening. Ventricular standstill was the terminal mechanism at death.

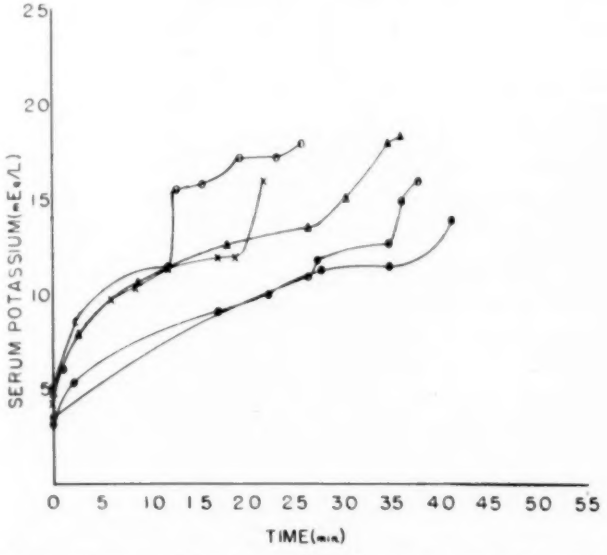

FIG. 4.—The serum potassium concentration is plotted against time in 5 experiments on normal dogs. The rise in serum potassium concentration is not linear. Note the tendency toward initial and terminal rapid increase in serum potassium concentrations. The geometric figures (X, 0, etc.) superimposed on the curves represent the points at which serum samples were taken for potassium determinations.

ing of the paroxysm but did not reach the control value; it continued to drop with the increase in intraventricular conduction time and slowing of the heart rate, dropping to zero with ventricular standstill or ventricular fibrillation.

The End-Point. The exact point at which death occurs was difficult to obtain in some dogs because of the relatively long periods (2 to 5 minutes) of slow idioventricular beats. When more potassium was given at this time there resulted an increase in the serum values because of the low blood pressure and poor circulation. The estimation of the serum concentration from the heart blood at the time of death would probably also give an abnormally high value for similar response.

Discussion. *Tolerance of the Normal Dog to Potassium.* From the evidence in the literature, as well as the experiments mentioned in this paper, it is fairly clear that the normal dog can tolerate relatively large doses of potassium given by the methods described. The ECG is a fairly good criterion for judging the varying degrees of the toxic effects of potassium; although extremely tall T waves occur during the initial stage of toxic effect, probably the most important clear-cut evidence of toxicity is the development of the initial grade of intraventricular block.

The recognition of the reversibility of potassium effects is of considerable importance, as cessation of potassium administration, even at an advanced grade of toxicity, will result in a quick return to normal. This agrees with the work of Hald, who used the isolated rabbit heart, Winkler, Hoff and Smith, who used intact dogs[20], and Chamberlain, Scudder and Zwemer[2], who used cats. When the concentration of potassium in the serum rose to a toxic level and was allowed to fall again, the animal recovered completely. There is no evidence from these experiments that a cumulative effect occurs; the maximum effect is observed immediately following the administration of the cation and tends to disappear rapidly when its administration is stopped.

Tolerance of Infarcted Animals to Potassium. The presence of a small infarct (Grade 1) did not significantly decrease the tolerance to potassium from the standpoint of the lethal dose. However, there was a significant decrease in the level at which even the dogs with small acute infarcts developed intraventricular block as compared to the normal control animals (Table 2). A definite diminution in tolerance to potassium in animals with moderate or severe grades of acute myocardial infarction was indicated by the serum concentration at which they developed intraventricular block and the serum concentration at death. Animals with severe grades of myocardial infarction manifested electrocardiographic evidence of toxicity similar to the normals. However, the stages over which the changes were present were extremely short; for example, following the development of bundle branch block, ventricular tachycardia and ventricular fibrillation developed rather quickly. The T wave changes in the presence of myocardial infarction are frequently slight and are not dependable as an early evidence of potassium effects.

Relation of Observations in Experimental Animals to Clinical Problems. These findings would suggest that in the presence of a normal or relatively normal heart with normal renal function, slowly administered potassium is well tolerated. The margin of safety is wide. Experience obtained in humans also reveals a similar wide margin of safety. However, the above experimental data suggest that in the presence of moderate and severe grades of

acute myocardial infarction, the evidence of toxicity may occur at lower levels of serum potassium than with normals. This may well apply to patients with myocardial infarction and other types of heart damage.

Summary and Conclusions. 1. Myocardial infarction does not gravely affect tolerance to the intravenous administration of potassium chloride.

2. Isotonic potassium chloride (1.14%) was administered intravenously at a rate of 10 cc. per minute to normal dogs and to dogs with acute and chronic myocardial infarctions. Almost continuous ECGs and continuous blood pressure records were taken. Blood samples for electrolyte determination were collected immediately upon the development of significant ECG alterations.

3. Normal dogs died at serum concentrations of 13.9 to 18 mEq. per liter of potassium. Dogs with minor degrees of myocardial damage showed a lethal serum concentration similar to the normal. Dogs with moderate to severe grades of infarction, both acute and chronic, showed a significant decrease in the lethal serum concentration of potassium at death.

4. The development of intraventricular block, which is taken to be definite evidence of potassium toxicity, developed at a significantly lower level in all types of acutely infarcted dogs (Grade 1 to 4) as compared to the normal.

5. In 6 dogs with healed myocardial infarction there was no significant change from normal in the serum concentration at which intraventricular block occurred, but death occurred at a slightly lower concentration as compared to the normal control.

6. No correlation was evident between the quantity of potassium chloride administered per kg. of body weight and the serum level of potassium at death.

7. Following the administration of potassium to normal dogs the potassium effect was reversible up to the stage of a marked degree of bundle branch block and ventricular tachycardia.

8. With the method of potassium chloride administration used in these experiments, the rise in serum potassium concentration did not appear to be a linear function of time. The following sequence was usually seen: an initial rapid rise, then a tendency to plateau, followed by a terminal increase in slope.

9. The significance of these findings —tolerance to potassium with a normal cardio-renal system, evidence of toxicity at lower levels of serum potassium in the presence of acute infarction, and so on—relative to the clinical problem is discussed.

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BERNHEIM'S SYNDROME CONSIDERED IN THE LIGHT OF A FATAL CASE*

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It is well recognized that isolated failure of the left ventricle is of much more frequent occurrence than primary failure of the right ventricle, except in those clinical states where undue burden is placed on the right ventricle, as in mitral stenosis or in chronic cor pulmonale. It is also appreciated that right ventricular failure may be chiefly responsible for the signs and symptoms that may appear even when preceded by left sided failure. Since, as White[7] points out, the essential evidence of progressive weakness of either ventricle is congestion behind that ventricle, it is interesting to speculate as to the mechanisms involved in those cases in which massive dilatation and hypertrophy of the left ventricle is manifested clinically by engorgement of the great veins and even anasarca, with little or no pulmonary congestion.

Bernheim[1,2] in 1910 observed a series of cases at necropsy in which, despite great hypertrophy and dilatation of the left ventricle resulting from hypertension and arteriosclerosis, the primary clinical finding was severe engorgement of the great veins in the absence of engorgement of the pulmonary vascular bed as might have been expected. His studies revealed that the greater portion of the cavity of the right ventricle had been markedly reduced by a greatly hypertrophied interventricular septum. By bulging convexly into the right ventricle, the septum had reduced the apical portion of the right ventricular cavity to a mere slit. The results of his findings led Bernheim to describe the syndrome which now bears his name.

There appears to be considerable doubt as to the actual importance of the concept of Bernheim in the explanation of this apparent paradox. In support of it are Fishberg's[5] observations on cases of long standing hypertension or aortic stenosis with severe systemic congestion which, at necropsy, showed quite remarkable obliteration of the right ventricular cavity by a bulging hypertrophied interventricular septum. He thinks, as we do, that such cases strongly support Bernheim's view. Henderson and Prince[6] cite experimental evidence in which perfusion of the left ventricle, with increased filling pressure and output, resulted in a diminished output from the right ventricle. They attribute this to displacement of the interventricular septum so as to interfere with right ventricular filling.

On the other hand, Evans and White[4] recently reported on a series of patients with grossly hypertrophied hearts in an effort to evaluate Bernheim's syndrome.

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Correlating the necropsy findings with the clinical history, they could find no instance of early signs or symptoms of isolated right sided failure. They concluded that they had yet to encounter any unquestionable cases of so-called Bernheim's syndrome. It was their belief that in all cases of right sided failure in the group studied, left sided failure with pulmonary hypertension had preceded systemic venous engorgement and was therefore etiologically related to the latter. We heartily agree with the concept that left sided failure is the most frequent cause of right sided failure, especially in hypertensive, arteriosclerotic disease. However, we do feel that there are some cases of hypertensive and arteriosclerotic disease or cases of aortic valvular disease in which, even early in the course of the disease, the signs and symptoms of right sided failure far outweigh, in clinical significance, the minimal, (if present at all) evidences of left ventricular insufficiency. Such is a case that we are about to report.

Case Report. J. G. (60,561), a 58 year old negro male was admitted to the hospital on September 9, 1948. He appeared moribund and a coherent history was unobtainable at first. Later, during a brief rally, he complained of generalized swelling of the entire lower half of the body. The anasarca was preceded by exertional and occasional nocturnal dyspnea which had antedated the edema by a 6 month period.

On physical examination, he appeared well developed; he was able to lie flat without any respiratory difficulty. His sensorium was clouded as to time and place. There was edema of the face and periorbital tissues. The neck veins were markedly distended and pulsating. The chest was symmetrical and expansion was equal. The percussion note was resonant throughout. Tactile and vocal fremitus were normal. The breath sounds were vesicular and no rales were heard.

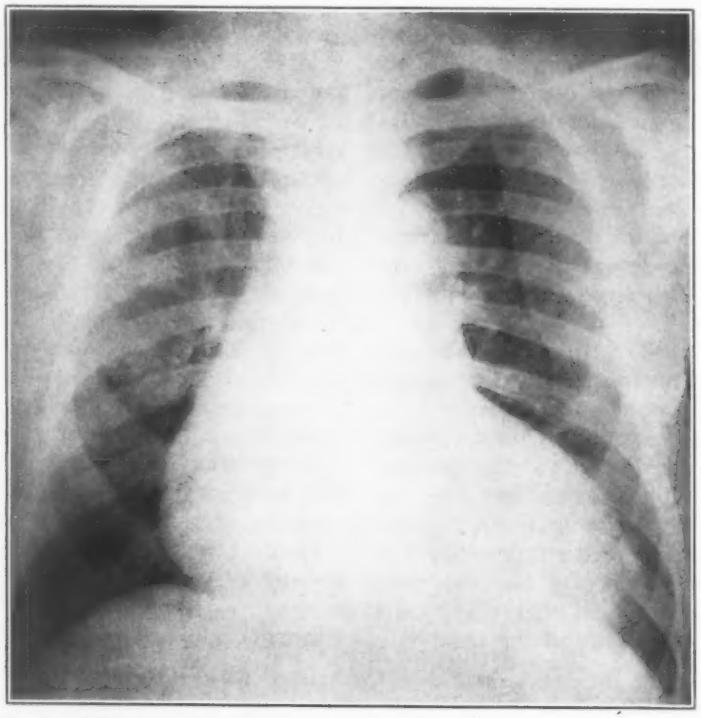

FIG. 1.—Massive left ventricular enlargement without pulmonary congestion (14 x 17 in.).

The heart was greatly enlarged to the left, the left border was located in the anterior axillary line. There was a coarse diastolic thrill felt over the entire precordium. The rhythm was regular sinus. The sounds were distant. A grade 4 diastolic murmur was heard maximally over the second interspace to the right of the sternum. P2 was not accentuated. The blood pressure was 138/62. The liver was enlarged and the hepatojugular reflex was positive. A 4+ pitting edema extended from the sacrum and genitalia downward to involve both legs in their entirety.

Laboratory studies on admission revealed a normal blood count. The blood urea nitrogen was 9.2 mg. per 100 cc. A random urine specimen showed no abnormalities. The standard Kahn test was reported as 2+, the Wassermann was negative. Roentgen-ray examination of the chest revealed marked enlargement of the heart to the left and right. There was slight dilatation of the arch of the aorta. The lungs were clear; there was no radiographic evidence of pulmonary congestion. An electrocardiogram indicated left ventricular hypertrophy and strain.

The patient was placed in an oxygen tent, rapidly digitalized with digitoxin, and mercurial diuretics were administered as indicated. Except for a brief rally, his course was rapidly downhill. There were no significant changes in physical findings—the lungs remained clear to percussion and auscultation throughout hospitalization and there was little or no reduction in the degree of anasarca. The patient died on the 9th hospital day.

Necropsy. (T. W. Simpson, M.D.). The pertinent findings were as follows: The right lung weighed 940 gm., the left 400 gm. The major pulmonary arteries and veins and the major bronchi revealed no abnormalities. On cut section, the right lung revealed discrete consolidation involving patches of lung tissue adjacent to the terminal bronchi. These areas were largely confluent in the right lower lobe which was greatly increased in consistency and appeared to be generally consolidated by the process. The heart was massively enlarged with preponderant hypertrophy of the left ventricle. The great veins of the neck and the right auricle were remarkably distended by coagulated blood. There did not appear to be much difference in the two atria as they were opened in the flaccid state; unfortunately, the walls of the atria were not measured. On section, the disproportionate hypertrophy of the left ventricle was even more striking, its wall measuring 3.5 cm. as contrasted to the 0.5 cm. thickness of the right ventricular wall. The interventricular septum was markedly hypertrophied and measured 3 cm. at its thickest portion. It projected into and obliterated the right ventricular cavity, which appeared as a mere slit. The mitral, tricuspid and pulmonic valves were normal. The aortic valve cusps, except for minimal calcification along the line of closure, were also normal in appearance. The commissures were not widened. The sinuses of Valsalva were markedly increased in depth. The coronary orifices were patent, as were the lumina of both vessels. Several atheromatous plaques were present along the course of each major branch. No actual occlusion was noted.

The aorta was greatly and symmetrically dilated in its proximal posterior portion. Upon opening it, there was noted a transverse tear with sharp cleanly cut edges extending transversely across and involving the intima and medial layers. The intima was separated at this point by approximately 0.5 cm. and was supported only by a thin layer of fibrous scar tissue and adventitia. There was a moderate degree of atheromatosis with plaques mottling the intimal surface of the proximal portion. There was no evidence of luetic aortitis. The arch of the aorta with its major branches and the descending aorta were each diffusely involved by an atheromatous process. There was no occlusion or anomaly of any major arterial branch.

The liver weighed 980 gm. On cut surface, there were noted contrasting areas of yellow parenchyma and dark red lobular centers. Microscopic studies revealed extreme engorgement of the central lobular area throughout with a wide zone of central necrosis.

Anatomic Findings. 1, Atherosclerosis of aorta with rupture of the inner coats; 2, Hypertrophy and dilatation of the heart; a, marked distention of right auricle by coagulated blood; b, wall of left ventricle measured 3.5 cm., of right ventricle 0.5 cm.; c, interventricular septum was markedly hypertrophied and measured 3 cm. at its thickest portion. 3, Bronchopneumonia, confluent; 4, Passive congestion of viscera, advanced, with central lobular necrosis of the liver.

Discussion. On admission, a diagnosis of hypertensive and coronary arteriosclerotic heart disease with severe congestive failure was recorded. The diastolic murmur of aortic origin was believed to represent "relative" aortic insufficiency; however, luetic

aortitis with insufficiency as the prime etiologic factor could not be readily excluded. The patient was treated vigorously for congestive failure. There was a total lack of response to the therapeutic measures administered. There was a peculiar lack of respiratory distress throughout this patient's hospitalization. The oxygen was administered, however, for the other aspects of his congestive failure.

The extreme degree of systemic venous congestion when contrasted with the absence of pulmonary engorgement was the most noteworthy clinical feature. We doubted, therefore, in the absence of signs of left sided insufficiency, including a pulmonic second sound which certainly was not accentuated, that right sided failure of this extreme degree was precipitated by prior insufficiency of the left ventricle. We have on frequent occasions in the past been impressed by the improvement in the manifestation of left sided insufficiency following right sided failure. However, almost invariably, there has remained unmistakable evidence of an incompetent left ventricle, either elicited by physical examination or made evident by mere observation of the patient. There was a total lack of such evidence in this case. We did not, however, definitely diagnose a Bernheim's syndrome, but it was suspected. We were content with the more generally accepted concept of prolonged, relatively mild, left sided insufficiency finally culminating in right sided hypertrophy and failure, with subsequent relief of pulmonary hypertension and its concomitant dyspnea dependent upon left ventricular failure.

It appears to us, however, in the light of the findings at necropsy, that a more logical explanation of the clinical findings would necessarily entail the concept of Bernheim. That the right ventricular cavity was seriously encroached upon was strikingly demonstrated to us at the necropsy table. There appeared to be very little resilience of the right lateral ventricular wall and it could not be stretched sufficiently to allow more than a few cc. of blood to enter the cavity. That this state of affairs could have resulted in a significantly diminished right ventricular filling resulting in a marked elevation of venous pressure behind the right ventricle appeared to us a likely explanation of the clinical state.

We cannot agree entirely with the rigid criteria which Evans and White[4] utilized in their evaluation of the Bernheim syndrome. We believe, for example, that the extreme systemic venous engorgement in the absence of any clinically significant degree of left sided failure present in our case was mainly dependent upon a practically obliterated right ventricular cavity, the wall of which measured slightly less than 0.5 cm. The liver, while severely engorged on microscopic examination, was also the seat of considerable fibrosis and much enlargement could not have been expected. The right lung was the seat of a confluent bronchopneumonia, not passive congestion. Its 900 gm. weight therefore is explainable on the basis of inflammatory consolidation.

It is further apparent to us that in cases of this type, even the presence of a mild degree of left ventricular insufficiency does not necessarily invalidate the concept of Bernheim. We agree that, in all probability, our patient's complaint of shortness of breath prior to admission here may have indicated a failing left ventricle. We believe that such failure was minimal, and its duration and severity do not appear to us to have been sufficiently significant to have been responsible for the subsequent course of events. Also it should be pointed out

that nocturnal dyspnea, in the presence of mild left ventricular insufficiency, may become manifest, as Dock[3] points out, as a result of mobilization of subclinical edema when the recumbent position is assumed. If this occurred in our patient, then the assumption that right sided "insufficiency" existed, or perhaps even antedated, the left sided difficulty would be tenable. We lean toward the concept of Bernheim in the unfolding of this clinical picture.

We would like to suggest that severe systemic congestion in cases of this type, where anatomic evidence of right ventricular cavity obliteration exists, may be explained quite readily on the assumption of interference with right ventricular filling. We do not believe that this concept necessarily becomes untenable when it can be shown that slight left sided failure co-exists. Certainly in those cases where the latter is absent, or questionable, as in our case, we believe that no better explanation of the clinical course has been suggested. We feel that the data presented in this case are in keeping with the concept of Bernheim and that the syndrome should not be dropped from the literature. Further recorded clinical reports with careful necropsy correlation will aid in the ultimate evaluation of this problem.

Summary. 1. A case of advanced hypertensive heart disease which clinically presented signs of systemic passive congestion out of all proportion to those of left ventricular insufficiency is presented.

2. It is believed that the eccentric left ventricular hypertrophy with the almost complete obliteration of the right ventricular cavity by the greatly hypertrophied interventricular septum offers a logical explanation of the clinical finding.

3. The presence of a mild degree of left sided failure does not in our opinion invalidate the concept of Bernheim when anatomic studies disclose severe encroachment upon the right ventricular cavity. Indeed some degree of left ventricular insufficiency should be expected, as the etiological factors operating to produce Bernheim's syndrome do so by their pathological effects on the left ventricle.

4. In our opinion a diagnosis of Bernheim's syndrome may be made when, in the presence of any etiological factor which causes a considerable burden on the left ventricle and results in its enlargement, the signs of systemic venous engorgement far outweigh in clinical significance the manifestations of left ventricular failure. When this clinical state can be explained at autopsy by the almost complete obliteration of the right ventricular cavity by an extremely hypertrophied interventricular septum, it is our contention that the concept of Bernheim has been fulfilled.

5. We believe that in the light of present knowledge the concept of Bernheim should be retained.

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SUDDEN AND UNEXPECTED NATURAL DEATH. III. SPONTANEOUS SUBARACHNOID HEMORRHAGE

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This study on spontaneous subarachnoid hemorrage is the third in a series of papers on sudden and unexpected natural death. The material upon which it is based comprises 2030 necropsied cases in the Borough of Manhattan division of the Office of the Chief Medical Examiner, New York City, from January 1st, 1937, to June 30, 1943.

The population of Manhattan, according to the 1940 census, was about 1,900,000 and predominantly adult, three-fourths of it over the age of 21 years. In addition, there are at any given time about 500,000 visitors in the city, mostly adults who spend their time in Manhattan. Also during the working day, and to a lesser extent at night, many adults from the other 4 boroughs of the city and from suburban communities add to the number of persons in this borough. Negroes comprise almost 16% of the fixed population, with all other colored races less than 1%.

The introductory paper[6] of this series, which was devoted to general considerations and statistics, may be briefly summarized. Each race contributed to the total of 2030 cases in almost exact proportion to its share of the population. Females, although comprising 51% of the population, made up about 19% of the total number of cases, a percentage corresponding to that of their sex in all medical examiners cases, autopsied and non-autopsied. Analysis of the racial distribution by sexes shows that there were relatively more colored than white females, and correspondingly fewer colored males than the total proportion of Negroes to white persons. There was a relatively large contribution from the first half-decade of life. These deaths occurred chiefly in the first year and were most often caused by respiratory infection. A small number of presumably natural deaths during the first year was encountered in which there was no satisfactory cause of death. The next 15 years contributed little. Beginning wih the age of 20 years, sudden and unexpected natural death became more frequent, but with a smaller percentage in each half-decade of the total deaths than the corresponding half-decade percentage of the total. Then there was a steady rise, with the greatest incidence of these deaths between 45 and 54 years. These two half-decades, as well as that of 40 to 44, and those over 55 years, reveal a percentage of sudden natural deaths greater than the corresponding half-decade's part of the population.

Of the diseases causing death, those of the heart and aorta were most numerous, comprising 45% of the total of 2030 cases. The respiratory system followed with 23%; the nervous system, 18%; the digestive tract and urogenital

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system together, slightly less than 10%. The remainder consisted of a miscellaneous category, three-fourths of which was supplied by estivo-autumnal malaria occasioned during the years in question by the direct artificial transmission of that disease in drug addicts. More than a third of the sudden natural deaths in the respiratory group (8% of the total) was caused by lobar pneumonia.

The second paper[12] of the series was devoted to coronary artery disease; it reported that two-thirds of all deaths from diseases of the heart and aorta were due to coronary arteriosclerosis. Relatively more deaths from the latter disease occurred in the older age-groups. In fact, over 30% of the entire material of 2030 deaths were furnished by it. In three-fourths of the sudden and unexpected deaths from coronary artery disease, coronary arteriosclerosis and not fresh thrombosis was responsible for the vascular occlusion. In the other fourth, thrombosis, not always fresh, of a coronary artery was encountered and an underlying sclerosis, usually of severe grade, was also present. Of the sudden deaths from coronary artery disease 90% occurred in white men.

Sudden and unexpected natural death in infants and young children, the fourth communication[13], is chiefly confined to boys and ordinarily is not encountered after the 3rd year of life. In fact, of 69 patents less than 5 years old, only 6 were older than 2 years. Negroes are included far beyond their share of the general population; this phenomenon probably is not purely biologically racial. Almost 80% of the deaths were attributed to respiratory disease. The role of the thymus is not yet to be clarified, and enlargement cannot be inculpated except for the very rare exception. There were instances in which the lethal factor could not be established; this was encountered in infants 4 to 8 months old.

The fifth report[14], classifying the causes of sudden and unexpected natural death by sex and age, evaluated fatal factors in certifying sudden death where autopsy is not feasible or cannot be performed.

In connection with sudden and unexpected death from spontaneous subarachnoid hemorrhage the first fact worth recording is its relative frequency (95 cases or 4.7% of the total 2030 necropsied cases of this series). This represents more than one-fourth (25.7%) of the 370 deaths from diseases of the nervous system. Spontaneous subarachnoid hemorrhage almost equals spontaneous cerebral hemorrhage into the basal ganglia as a cause of sudden natural death. Of the 95 subjects, 73 were white, and 22 Negroes (Table 1). The latter incidence represents 23.2% of the series, or about 1½ times the proportion (15.8%) of the black race in the population. Of the Negroes, 12 were males and 10 females; among the whites, there were 47 men and 26 women. The total sex distribution was 59 men and 36 women (1.7:1). This disparity would seem to favor the male sex if one considers that in the general population the ratio of the sexes is close to 1:1; but since the proportion of men to women in medical examiner's cases is 4:1, women actually contribute more than twice their expected share of this disease.

Although recent papers call attention to the fact that spontaneous subarachnoid hemorrhage is not a disease of the young, contrary opinions are sometimes heard on hospital wards. Hence, it is of interest to report the wide age distribution of the condition. In addition to single cases in the 1st, 3rd, and 4th half-decades, the range is between 20 and 69 years. Beginning at 20 to 24 years, and advancing by 5 year periods, the incidence of death

TABLE 1.—AGE, RACE, AND SEX DISTRIBUTION OF 95 CASES OF SPONTANEOUS SUBARACHNOID HEMORRHAGE

	White			*Negro*			*Total*			
Age, Years	*M*	*F*	*Total*	*M*	*F*	*Total*	*M*	*F*	*Total*	
< 5	0	0	0	1	0	1	1	0	1	1.0%
5—9	0	0	0	0	0	0	0	0	0	
10—14	1	0	1	0	0	0	1	0	1	1.0
15—19	1	0	1	0	0	0	1	0	1	1.0
20—24	2	2	4	0	0	0	2	2	4	4.2
25—29	4	1	5	0	3	3	4	4	8	8.4
30—34	8	4	12	2	1	3	10	5	15	15.8
35—39	9	3	12	2	1	3	11	4	15	15.8
40—44	3	3	6	4	2	6	7	5	12	12.7
45—49	12	3	15	1	2	3	13	5	18	18.9
50—54	4	3	7	2	1	3	6	4	10	10.7
55—59	2	2	4	0	0	0	2	2	4	4.2
60—64	1	3	4	0	0	0	1	3	4	4.2
65—69	0	2	2	0	0	0	0	2	2	2.1
Total	47	26	73	12	10	22	59	36	95	100%

from this cause is 4, 8, 15, 15, 12, 18, 10, 4, 4, and 2. Expressed as percentages on the basis of the 95 cases in the series, the values are only slightly higher. The true contribution of each age-group to the disease can be evaluated only if the proportion of the age group in the general population is known. This is illustrated in Fig. 1. There are disparities in disease-contribution and population-share, the greatest furnished by the years between 45 and 49, which, with 8.1% of the population, yielded 15.8% of the deaths from spontaneous subarachnoid hemorrhage, a ratio of more than 2 to 1. This ratio is only 0.5 to 1 for the half-decade beginning at 20 years; at 25 years, 0.9; 30 years, 1.6; 35 years, 1.6; 40 years, 1.5; 45 years, 2.3; 50 years, 1.5; 55 years, 0.8; 60 years, 1.0; 65 years, 0.7 to 1. Fatal spontaneous subarachnoid hemorrhage, then, is a condition of maturity, occurring relatively most commonly in late young manhood and in middle age, especially in the latter period.

The prominence of spontaneous subarachnoid hemorrhage among the diseases of the nervous system producing sudden or unexpected natural death is striking. This is especially true when it is recalled that spontaneous cerebral hemorrhage, cerebral thrombosis and embolism, cerebral infarction, neoplasm, and tuberculous and suppurative meningitis are included in this category. More than one-fifth (22.2%) of nervous system maladies in the half-decade between 20 and 24 years in these studies is caused by spontaneous subarachnoid hemorrhage. For the succeeding half-decades, the percentages are as follows: 26.6% (25 to 29 years), 38.5% (30 to 34), 36.6% (35 to 39), 21.4% (40 to 44), 25.4% (45 to 49), 20.4% (50 to 54), 14.3% (55 to 59), 14.3% (60 to 64), and 11.1% (65 to 69 years).

In the majority of cases the source of the fatal spontaneous subarachnoid hemorrhage was the rupture of a saccular aneurysm of a cerebral artery, corroborating the findings of Martland[9] and of others[10,15]. Most of the aneurysms encountered in the present series were found at the bifurcation of the cerebral arteries and were of the type described by Forbus[3] who considered that the lesions are acquired and arise from the combination of a focal weakness in the vessel wall dependent on a congenital defect in the muscularis at points of bifurcation, and

subsequent degeneration of the internal elastica due to continued overstretching by the force of blood pressure. Based on an embryologic study of the cerebral arteries, Bremer[1] showed that some aneurysms, including those frequently encountered on the anterior communicating artery as well as the rarer ones which are occasionally seen as saccular protrusions along the course of some of the cerebral arteries, are actually congenital in their development and already existent in the embryo and at birth. He also emphasized that the anterior communicating artery is rarely a single vessel, but actually a plexus of vessels corresponding to the original capillary plexus from which it is developed and that developmental disturbances incident to the formation of these vessels give rise to the saccular aneurysms. He, as well as others[4], did not accept Forbus' explanation that aneurysms develop at points of bifurcation primarily because of congenital muscular defects at these sites. Nevertheless, the mechanism postulated by Forbus explains most satisfactorily the location and character of most of the aneurysms encountered in this series and also their relatively late appearance.

Simpson[15] also pointed out that the aneurysms should not be characterized as congenital, since they are not present at birth, and suggested that they be designated as developmental. He reported finding an aneurysm in 78% of cases of spontaneous subarachnoid hemorrhage and recommended the use of stereoscopic dissecting glasses and perfusion of the vessels in cases where the source of bleeding is not easily found. Whether the aneurysms are considered congenital or acquired on a congenital basis, post-natal circumstances may enhance their production, size, and probability of rupture. Aneurysms were often found associated with other anomalous formations of the circle of Willis.

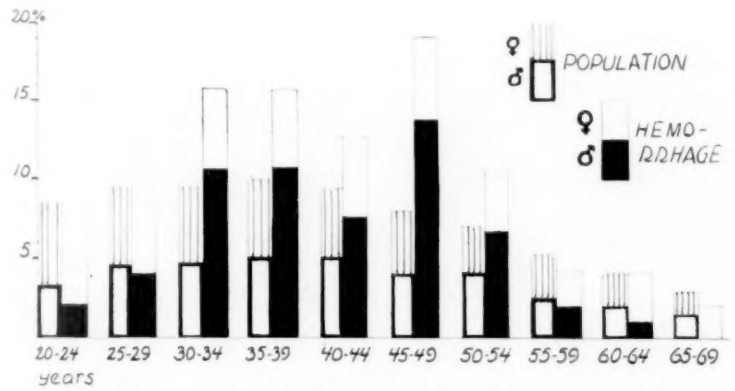

FIG. 1. Percentage distribution, by half-decades and sex, of population of Borough of Manhattan, New York City (1940 Census), and of 95 cases of spontaneous subarachnoid hemorrhage (1937-1943).

Carmichael[2] in studying the cerebral vessels observed defects in the elastic tissue concentrated in the internal elastic membrane, as well as in the medial muscle, and was of the opinion that the deficiency of one could be resisted by adequacy of the other. He concluded, however, that "not one of these conditions, by itself and in its

ordinary form, provides a sufficient basis for the formation of a saccular cerebral aneurysm. The cause of these aneurysms must therefore be sought in some specialized lesion or in some peculiar combination of the common lesions." He pointed out that in atheromatous vessels the dense fibrous tissue of the intimal plaque tends to prevent aneurysmal development despite loss of elastic and muscle layers. Simpson[15] cautioned against misinterpretation of aneurysms in later life, pointing out that atherosclerosis may occur in saccular lesions but that the lesions are developmental rather than degenerative. He mentioned the fact that atheromatosis of the cerebral arteries may lead to bulbous and fusiform but not to saccular dilatation and indicated that atheromatous enlargements occur "along the course of affected vessels rather than from the stems of their branches", as do the saccular aneurysms with which this paper is concerned. In most cases the aneurysms were single. In a recent case, not included in this series, 5 aneurysms of varying size were found on and in the vicinity of the circle of Willis. The largest, on the anterior communicating artery, ruptured to produce the fatal subarachnoid hemorrhage.

In two-thirds (64) of the subjects, the ruptured aneurysm was identified; in 22 cases the source of the subarachnoid hemorrhage was not located after dissection, and in 9 instances the presence or absence of an aneurysm was not determined because of incomplete dissection. See Table 2, in which it is seen that the percentage of cases in which a ruptured aneurysm was not found corresponds, in general, with that reported by other observers. The anterior communicating artery, as the site of location of the aneurysm, leads with 29.7% in this series; that vessel was involved in 28.2% of the cases reported by Taylor and Whitfield[16], and in 22.2% by Magee[7]. This latter discrepancy can

TABLE 2—SITES OF RUPTURE OF INTRACRANIAL ARTERIAL ANEURYSMS. TOTAL OF 196 CASES, INCLUDING 95 OF THE AUTHORS

	Helpern Rabson		*Magee*		*Taylor et al.*		*Total*	
Site of Rupture—Artery	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*
Ant. cerebral, rt.	4	4.2	5	8.3	4	9.3	13	6.6
" " , lt.	2	2.1	2	3.7	4	9.3	8	4.1
Middle " , rt.	11	11.6	7	12.0	4	9.3	22	11.3
" " , lt.	12	12.7	5	8.3	2	4.7	19	9.7
Ant. communicating	19	20.0	8	13.9	9	20.9	36	18.4
Int. carotid, rt.	3	3.2	0		0		3	1.5
" " , lt.	0		0		0		0	
Post. communicating, rt.	2	2.1	2	3.7	1	2.3	5	2.6
" " , lt.	0		0		3	6.9	3	1.5
Post. cerebral, rt.	2	2.1	2	3.7	0		4	2.0
" " , lt.	0		0		1	2.3	1	0.5
Sup. cerebellar, rt.	0		0		0		0	
" " , lt.	1	1.0	0		0		1	0.5
Basilar	4	4.2	4	6.9	2	4.7	10	5.1
Vertebral, rt.	1	1.0	0		1	2.3	2	1.0
" , lt.	1	1.0	1	1.7	2	4.7	4	2.0
Ant. inf. cerebellar, rt.	0		0		0		0	
" " " , lt.	0		0		0		0	
Post. " " , rt.	1	1.0	0				1	0.5
" " " , lt.	1	1.0			0		1	0.5
Aneurysm not found	22	23.2	15	25.8	8	18.6	45	23.0
" " " looked for	9	9.6	7	12.0	2	4.7	18	9.2
Total	95	100	58	100	43	100	196	100.0

be in part explained by reference to the diagram in Fig. 2 in which the sites of demonstrable rupture of an aneurysm in our cases are indicated. From a consideration of the diagram it is easily seen that it is often difficult to determine which artery is at fault when a ruptured aneurysm is found at or about the junction of the anterior cerebral and anterior communicating arteries. A similar dilemma is met with at the junction of the anterior and middle cerebral arteries with the internal carotid and posterior communicating vessels. That this is the likely explanation for the variations in values ascribed to these arteries as the sites of the aneurysms in the 3 series is readily understood if one adds the percentages of cases in each series, taking only anterior cerebral, middle cerebral, and anterior communicating arteries. Magee's[7] data add up to 75%, as do Taylor and Whitfield's[16] and those presented in this paper. Another observation, therefore, is that about three-fourths of the ruptured aneurysms are to be found in the anterior portion of the circle of Willis.

The ratio of right to left sides in the anterior area is 1.7:1 in Magee's[7] report; 1.3:1 in that of Taylor and Whitfield[16]; and 1.1:1 in our cases. Taking all arteries but the anterior

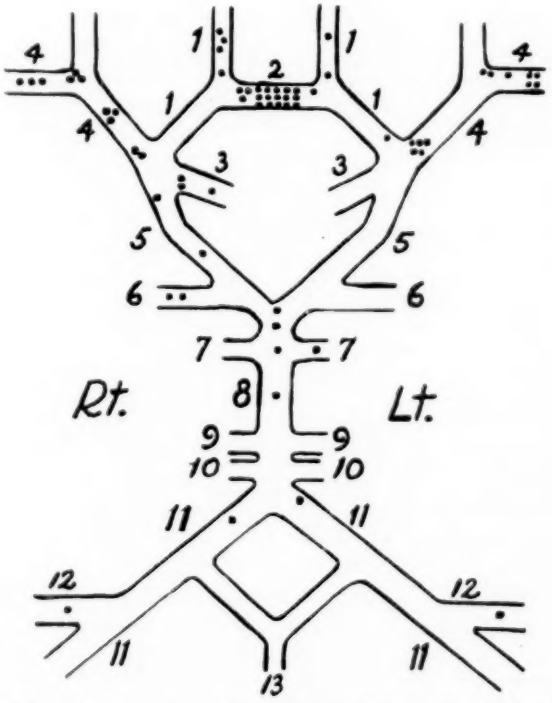

FIG. 2. Diagram of 13 cerebral arteries showing sites of 63 of the ruptured aneurysms. It is emphasized that locations of aneurysms along the course of vessels away from bifurcations are more apparent than real; they are so drawn because of lack of more specific information in the protocols.

(1, Anterior cerebral. 2, Anterior communicating. 3, Internal carotid. 4, Middle cerebral. 5, Posterior communicating. 6, Posterior cerebal. 7, Superior cerebellar. 8, Basilar. 9, Internal auditory. 10, Anterior inferior cerebellar. 11, Vertebral. 12, Posterior inferior cerebellar. 13, Anterior spinal).

communicating and basilar vessels, the ratio of right to left, in the 3 series, is 2:1, 0.8:1, and 1.4:1, respectively; for all 3 series combined, it is 1.4:1.

The most frequent anatomic variety of hemorrhage from rupture of a cerebral arterial aneurysm is the diffuse subarachnoid type. In this variety there is rapid extravasation and accumulation of blood on the under surface of the brain, with extension along the fissures of Sylvius and into the cisterna magna and 4th ventricle. Death is usually very rapid, presumably from compression of the medulla. In this type the aneurysm, if present, can readily be found at one of the usual sites on the cerebral arteries. Similar rapid subarachnoid extravasation of blood can occur from a ruptured cerebral artery and the bleeding point may not be demonstrable despite the most careful dissection.

Examples of rapid diffuse subarachnoid hemorrhage are illustrated in the following 2 cases.

Case Reports. CASE 1. A sturdy Coast Guard chief petty officer, 26 years old, came home on week-end leave late Saturday afternoon. Before, during, and after dinner he drank several whiskey highballs. He and his wife retired about midnight. Shortly thereafter, during coitus, he made gurgling noises, and became unconscious. The wife rushed from the house to 'phone for an ambulance. On her return, several minutes later, the man was dead. Autopsy revealed massive spontaneous subarachnoid hemorrhage arising from an easily identifiable aneurysm of the circle of Willis.

CASE 2. A 14 year old schoolboy was annoying the apartment house doorman by rollerskating in the lobby. When the lad refused to quit, the man slapped him across the face. The boy collapsed and died within a few minutes. At necropsy a large subarachnoid hemorrhage was found; the exact source of the bleeding could not be demonstrated. An aneurysm was not found, but there was an anomalous asymmetric development of the cerebral arteries, which were extremely delicate. There was no physical evidence of trauma. The doorman had been taken into custody but was released when the autopsy findings were disclosed.

In the search for the aneurysm, the stripping of the meninges and the exposure of the vessels is best carried out while the blood clot is still soft and easily washed away. The vessels are much more satisfactorily revealed by this method. Hardening the brain in formalin prior to dissection makes the latter procedure more difficult and the results are not as satisfactory. Besides, in the medicolegal autopsy, it is important to determine promptly the nature and source of an intracranial hemorrhage; this is especially indicated in cases in which death has occurred during or following an altercation, and an alleged assailant has been arrested pending the outcome of the autopsy.

There is a less common form of subarachnoid hemorrhage following the rupture of an aneurysm of the middle cerebral artery high in the fissure of Sylvius. This results in the production of a localized hematoma between the island of Reil and the overlying cortex. On casual examination, section of the brain through such an area of hemorrhage may erroneously suggest an intracerebral rather than a subarachnoid location.

In a few instances subarachnoid hemorrhage may distend and rupture the arachnoid, extending into the subdural space. Such subdural hemorrhage may be confused with the more common traumatic variety. Sometimes an aneurysm of the internal carotid artery, and rarely of the middle cerebral, breaks directly into the subdural space, producing subdural without subarachnoid hemorrhage[6]. Obviously, on such occasions failure to search for an existent aneurysm may lead to the erroneous diagnosis of traumatic subdural hemorrhage.

Aneurysms of the cerebral arteries

during their development may become embedded in the adjacent cerebral substance. Subsequent rupture, often preceded by confusing premonitory symptoms without immediate loss of consciousness, may give rise to intracerebral bleeding with extension into the ventricles, followed by spread to the subarachnoid space. At times, the latter is uninvolved and remains free of hemorrhage. Such cases may bleed slowly, and, until the hemorrhage breaks into the ventricular system and subarachnoid space, the spinal fluid remains free of blood. Intracerebral hemorrhage, occurring at sites other than the basal ganglia, is usually the result of rupture of such embedded aneurysms.

The following is an example of delayed death with confusing premonitory symptoms resulting from the slow rupture of an embedded cerebral artery aneurysm.

Case Reports. A 58 year old business executive, who was known to have hypertension but was otherwise well, developed a severe headache during a luncheon following a business meeting. He telephoned his physician and mentioned to him that he had never had such a severe headache before. His doctor advised him to come to his office immediately for an examination. Unaccompanied, the patient hailed a taxicab and gave the driver the address of the doctor's office which was in a professional building about a mile away. When the taxi arrived at its destination, the patient was conscious but disturbed and confused, and he was unable to state his doctor's name after entering the building, where he was not recognized by the attendant then on duty. Because of his excitement and mental confusion he was taken to the office of a psychiatrist on another floor in the same building. The psychiatrist diagnosed the patient's condition as alcoholism and instructed the taxi driver to take him home, which he did. By the time the cab arrived at the apartment house, the patient was stuporous. The driver called the superintendent of the house and told him the diagnosis that had been made by the psychiatrist. Believing the patient to be drunk, they half carried the now helpless man to his apartment where they laid him down on the floor in the belief that he would sleep off his "drunken condition". About 2½ hours had elapsed between the time the patient telephoned his doctor and the time that he was carried into his home. Several hours later, the superintendent looked in on the man, and, finding him now deeply unconscious, summoned a neighborhood doctor, who arrived shortly before the patient died. Not until after the patient's death did his own physician finally learn what had happened. The case was reported to the medical examiner's office for investigation, and because of the unusual circumstances an autopsy was performed. This revealed a ruptured saccular aneurysm, 6 mm. in size, of the circle of Willis located on the left anterior cerebral artery in the vicinity of the anterior communicating vessel; the aneurysm was embedded in the left frontal lobe. The rupture at first produced a localized intracerebral hemorrhage which in turn ruptured into the lateral ventricle and into the subarachnoid space. The premonitory symptoms of the rupture were first headache, then mental confusion and speech aphasia, and lastly unconsciousness. The business associates of the deceased stated that he had not taken any drinks because of the headache.

It is appropriate at this point to mention the paper of McDonald and Korb[8] which included an extensive review of 1125 cases of ruptured and intact aneurysms of arteries at the base of the brain. The cases, seen at autopsy or operation, began with that reported by Morgagni in his "De Sedibus et Causis Morborum", 1761. Spontaneous subarachnoid hemorrhage, per se, however, was not discussed.

What individuals suffer from spontaneous subarachnoid hemorrhage, and under what circumstances? The occupations most represented in the 95 cases were that of "housewife", 17 times; no record, 13; laborer, 12; mechanic, 7; clerk, engineer, and salesman, 4 each; actress, dishwasher, engineer, houseworker and seaman, 3 each; janitor, stationary fireman and telephone operator, 2 each. Most of the remaining 18 were in manual occupations of many varieties.

The place where death or collapse took place is of interest. Death came at home in 43 instances (45%), with 10 of these in bed. In 8 cases a bed other than that of deceased, usually in a hotel or rooming house, was recorded as the place of death. Hemorrhage occurred 14 times while the subjects were walking on the street or in the park, 7 times at relatively arduous, and once at sedentary work, 4 times in public places and 4 times while visiting friends.

The exact circumstances under or during which the disease manifested itself is recorded in only a minority of cases. In about two-thirds of the total of 95 cases occurring at home, in strange beds, or on the street, these circumstances were elicited only occasionally. An altercation was reported or suspected in 12 instances, 5 taking place on the street. In these cases, either no physical evidence of trauma or only slight marks of traumatic injury were found, indicating that trauma was not a factor in causing the hemorrhage. A ruptured aneurysm was found in 3 of these 12 cases; in 7 instances an aneurysm could not be found after dissection; in 2 cases presence or absence was not recorded; in a few cases, cerebral arterial anomalies were observed.

The role of acute alcoholic intoxication is important in reviewing death from spontaneous subarachnoid hemorrhage, particularly because of the active hyperemia of the brain induced by alcohol. Whether the added burden thrown upon the cerebral circulation, and therefore upon cerebral artery aneurysms, by the hyperemia is a factor in rupture is worthy of consideration. Unfortunately, chemical examination of the brain was not performed in 52 cases. In the remaining 43 cases the positive findings* outnumbered the negative results by 3:2; with a 3 + amount of alcohol as compared to 2 + alcohol, in the same ratio of 3:2. The findings in 2 instances were recorded as 4 +. In the instances of actual or suspected altercation, chemical examination was not done in 1 of the 2 cases in which the presence of aneurysm was not stated; in the other case, there was 3 + alcohol. The 3 cases with ruptured aneurysms were strongly positive. In only one of the 7 cases in which the source of bleeding could not be found after dissection, that of a boy of 14 years, was the brain not examined for alcohol; all the others had findings of 3 +.

Death took place within "a few minutes" after the onset of symptoms in 25 (25%) of the 95 cases. An interim period of less than half an hour was reported 6 times, less than 1 hour in 6 subjects, a "few hours", 9 times, and less than 6 hours, 8 times. If there was not fatal termination within 6 hours (57 cases, or 60%), life usually continued for at least several days (19 cases). In one instance the patient survived for 22 days.

Summary. In a total of 2030 cases of sudden and unexpected natural death necropsied between 1937 and 1943, there were 95 cases (4.7%) of spontaneous subarachnoid hemorrhage. This represented 25.7% of the 370 fatalities from diseases of the nervous system. There were 59 men and 36 women; 22 were Negroes.

With 3 exceptions from the earlier years of life, the cases fell between the years 20 and 69. Spontaneous subarachnoid hemorrhage was found as a cause of death relatively most commonly in late young manhood and in middle age.

In the majority of instances rupture of a cerebral arterial aneurysm was the source of the lethal bleeding. In two

* 1 + alcohol, 0.02-0.10%; 2 +, 0.11-0.25%; 3 +, 0.26-0.40%; 4 +, 0.41-0.60%.

thirds of the cases the aneurysm was identified, whereas in almost one-fourth the source of the hemorrhage could not be located. This experience duplicated that of other observers. Similar corroboration was noted for the fact that 75% of the aneurysms were on the anterior and middle cerebral, and anterior communicating arteries. The right side was predominant, 1.4 to 1.

Diffuse subarachnoid hemorrhage covering the base of the brain was the most common anatomic variety. Less commonly there was a localized subarachnoid hematoma over the island of Reil, or a hemorrhage into the subdural space either directly or by a break through of a hemorrhage in subarachnoid space. In a few instances, an aneurysm embeds itself during development in the adjacent cerebral substance; when such an aneurysm bursts, intracerebral hemorrhage may result, usually with but sometimes without extension into the subarachnoid or ventricular spaces. Not infrequently there are confusing premonitory clinical phenomena without immediate loss of consciousness.

All occupations were represented. Death or collapse took place in 45% of the cases at home, with slightly less than one-fourth of these in bed, in 15% on the street or in the park. The exact circumstances immediately preceding death or collapse were elicited only occasionally in the two-thirds of the 95 cases occurring at home, in strange beds, or on the street. Alcohol determinations on the brain were carried out in 45% of the total; positive findings were recorded in three-fifths of these.

It is to be emphasized that dissection of the cerebral arteries should be done as soon as the brain is removed from the skull, while the blood clot is soft and easily removed by washing. The nature and source of hemorrhage on the surface or in the substance of the brain should be determined promptly, especially in cases of suspected criminal violence in which a suspect has been taken into custody. The usual method of waiting for formalin hardening of the brain is not practicable.

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SICKLE CELL ANEMIA IN THE WHITE RACE:
Study of a Family with a Review of Genetic Theories

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Sickle cell anemia is predominantly a disease of the Negro race, but both sicklemia and sickle cell anemia have occurred in persons in whom there can be no reasonable suspicion that admixture of Negro blood has occurred. Fourteen well documented cases of sickle cell anemia occurring in white individuals have been recorded[2-4,6-9,12,14,15] (Table 1). Several other cases have been reported in which family studies were not done and in whom the possibility of the presence of Negro blood is strong. There is no doubt that both sicklemia and sickle cell anemia are inherited, but none of the prevalent genetic theories is applicable to all the reported cases. Further accumulation of data is desirable to establish the genetic pattern of the

TABLE 1.—A REVIEW OF ACCEPTED CASES OF SICKLE CELL ANEMIA IN THE WHITE RACE

No.	Author	Year	Sex	Age	Nationality of Parents	Father's Blood	Mother's Blood	Others in Family
1.	Cooley and Lee[1]	1929	M	4	Greek	Normal	Sickled	1 normal sibling 1 brother sickled
2.	Rosenfeld and Pincus[5]	1932	M	9	Italian	Dead—cause unknown	Sickled	Maternal grandmother, aunt and patient's sister sickled
3.	Clarke[2]	1933	M M	11 3	Sicilian	Normal	Normal	3 normal siblings
4.	Cooke and Mack[3]	1934	F M	3½ 10 mo.	American (Kentucky)	Sickled	Normal	4 siblings with palpable spleens, no sickling
5.	Weiner[4]	1937	M	8	Sicilian	Normal	Normal	2 brothers have sickling, anemia, splenomegaly, maternal grandmother and 1 sister normal
6.	Johnson and Townsend[6]	1937	M	5	Greek	Dead—cause unknown	Normal	3 siblings not examined
7.	Hayden and Evans[7]	1937	F F	21 15	Sicilian	Dead—blood dyscrazia, anemia & jaundice	Normal	1 brother sickled, 4 other siblings normal
8.	Greenwald and Burrett[8]	1940	F F	22 9	Sicilian	Dead—heart disease	Sickled	2 normal brothers, maternal aunt and cousin sickled
9.	Mallory[9]	1941	F	20	Italian	Refused examination	Normal	2 normal siblings
10.	Ogden[12]	1943	M	9	Spanish	Normal	Sickled	Maternal grandmother died 35, anemia, maternal aunt died 14, anemia, 2 normal siblings
	Guyton and Heinle	1949	F	19	Sicilian	Dead—Caoflung	Sickled	Brother, maternal aunt and 2 uncles sickled; 4 siblings dead, 1 with anemia and jaundice, cardiac enlargement, splenomegaly

disease. This report describes the occurrence of sickle cell anemia in a white individual in whom all available relatives were studied for the sickling trait and sickle cell anemia.

Case Report. Case 1. L. N., a 19 year old Sicilian girl, was first admitted to Lakeside Hospital in April, 1949, complaining of fever, severe leg pain, dark urine and yellow eyes. She had been treated with liver and iron since the age of 5 years for "splenic anemia." Her first known hemolytic crisis occurred 2 years previous to this admission with back and shoulder pain and dyspnea. She was found to have anemia, splenomegaly, and an elevated icterus index. The diagnosis of sickle cell anemia was first made in 1948, one year before this admission. A splenectomy was performed at another hospital following her 5th hemolytic crisis. The rationale for the splenectomy is not apparent. The patient was a poorly developed, jaundiced, young white female with no negroid characteristics. There were no leg scars and no adenopathy. Laboratory findings (Table 2) were as follows: Erythrocytes 2.97 million per cmm., hemoglobin 9.8 gm. per 100 ml., hematocrit 31%, leukocytes 21,000 per cmm., reticulocyte count 23%, mean corpuscular hemoglobin 32 mcmcgm., mean corpuscular volume 103 cu. microns, mean corpuscular hemoglobin concentration 32%. The platelets were slightly increased in number, as estimated from cover slip films. There was 100% sickling of the erythrocytes in 24 hours in a paraffin-sealed wet preparation. Urine urobilinogen was 5 Ehrlich units in a 2 hour specimen. Total serum bilirubin was 4.42 mg. per 100 ml. of which 2.64 mg. was direct and 1.78 mg. indirect. Erythrocyte fragility began at 0.46% saline and was complete at 0.28%. Roentgenograms of the gall bladder revealed 4 laminated calculi, and of the tibia and fibula revealed a moderate degree of osteoporosis with loss of the normal trabeculation of the medullary canal in the lower third of the tibia. Her symptoms subsided spontaneously with symptomatic treatment and bed rest. Since this time she has been admitted twice with hemolytic crises. Study of the 9 available members of her family revealed the sickling trait in the patient's mother, brother, a maternal aunt and two maternal uncles, none of whom had anemia or history or physical findings suggestive of sickle cell anemia. Her maternal grandfather, an uncle, an aunt, and a cousin were found to be normal (Fig. 1 and Table 2).

TABLE 2. LABORATORY FINDINGS OF THE PATIENT, L. N., AND OF HER FAMILY

	Generation	*RBC*	*Hgb.*	*Het.*	*WBC*	*Retic.*	*MCH*	*MCV*	*MCHC*	*Platelets*	*% Sickling in 24 hours*
L. N.	III	2.97	9.8	31%	21,000	23.0%	32	103	32	sl. increased	100
V. N.	III	4.35	11.2	41%	12,000	0.8%	25.7	94.2	27.4	normal	84
G. N.	II	4.43	12.3	43%	12,400	0.9%	27.8	98	28.3	normal	49
A. F.	II	4.93	16.4	50%	8,100	0.6%	33.2	102.5	32.5	normal	72
N. F.	II	4.83	14.9	49%	8,600	0.4%	30.9	101	30.4	normal	67
J. F.	II	5.15	16.1	51%	5,150	0.4%	30.3	100	30.3	normal	none
G. F.	I	4.73	15.0	48%	6,650	1.0%	31.7	101	31.3	normal	none
T. F.	III					0.7%					none
L. B.	II	3.83	11.9	39%	5,650	0.9%	31.1	102	30.5	normal	none
M. C.	II	4.62	14.4	44%	5,150	2.0%	31.2	95.3	32.7	normal	81

Discussion. It has been a commonly, but as we believe unjustifiably, accepted hypothesis that sickle cell anemia is due to a single, completely penetrant, dominant factor. Wintrobe[16] stated that it is probably transmitted according to the Mendelian laws of

heredity as a dominant characteristic. This explanation cannot be applied to the cases studied by Clarke[2], and Weiner[15] recorded in Table 1. In 1947, Neel[10] presented several plausible explanations of the method of transmission of the disease. One possibility is that there are two distinct genetic types of sickle cell anemia, one due to a recessive factor, and one due to a dominant factor. Another is that the responsible gene is not always completely penetrant, and is not expressed in all individuals having the gene so that their blood is normal. Still another possibility suggested by Neel[10] is that there is a gene in Negro populations which when heterozygous results in sicklemia and when homozygous results in sickle cell anemia, a situation analagous to that which exists in thalassemia. More recently Neel[11] has published data from a study of sickle cell anemia in Negro families which adds support to this last theory. A study of the parents of patients with sickle cell anemia revealed the sickling trait in both parents in all 13 instances studied, and in the only parent studied in 16 others. Beet[1], after studying the sickling trait in a Bantu tribe, stated that sickle cell anemia occurs only in homozygotes. Pauling *et al.*[13] recently found that in sickle cell anemia, all of the hemoglobin has an abnormal molecular structure, whereas, in the trait form of the disease, both normal and abnormal hemoglobin are present in a constant ratio of approximately 50:50. Using the homozygous-heterozygous theory proposed by Neel, he suggested that in sickle cell anemia both alleles responsible for the structure of the hemoglobin molecule are abnormal (homozygous), producing 100% abnormal hemoglobin, while in sicklemia only one allel is abnormal (heterozygous), producing only 50% abnormal hemoglobin.

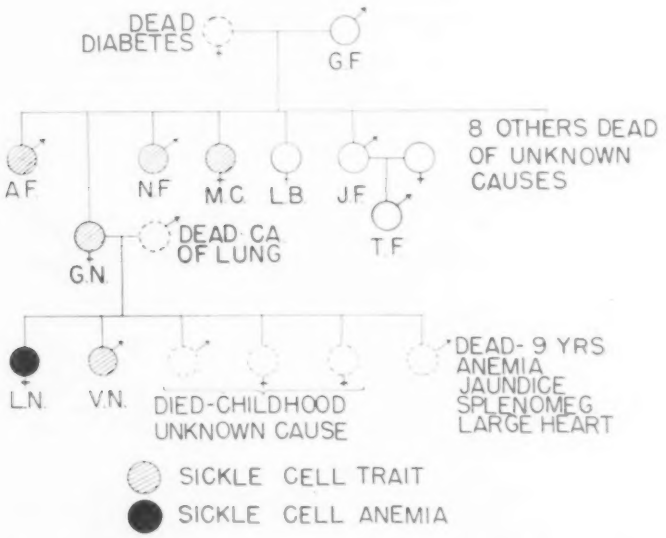

FIG. 1.—Case L. N. Sickling incidence in 9 members of her family.

If the unmodified theory of the homozygous origin of sickle cell anemia is accepted, however, it is necessary to assume, as indicated by Neel[11] and Dameshek[3], that in the

cases in which one or both parents did not exhibit the sickling trait (1, 3, 4, 5, 10 in Table 1), the technique of demonstrating sickle cells was faulty. Such an assumption does not appear to be entirely warranted. Furthermore, it is hazardous to base any genetic study on data obtained from the Negro race because of the well known frequency of impaternity in this race. Nevertheless, the heterozygous-homozygous theory can probably be applied to sickle cell anemia and sicklemia with the modification that instances in which one or both parents of a sickle cell anemia patient show no sickling are due to incomplete penetrance of the trait-producing gene in the parents.

It is unfortunate that in our case the patient's father was not available for study, so that more support could have been given to one of the possible genetic theories. There can be no doubt that there is a hereditary factor responsible for the sickling trait which was present in some of the maternal members of this family. No members of the family had any negroid characteristics and there was no known admixture of Negro blood in the family history. However, as in all reported cases of sickle cell anemia in the white race, which occur with only one exception[3] in individuals from the region around the Mediterranean Sea, the possibility of remote admixture with Negro blood cannot be absolutely excluded.

Summary. A case of sickle cell anemia in a white Sicilian girl is presented with a family study showing that the sickling trait was present in 5 of 9 available members of her family. Possible theories of the genetics of sickle cell anemia are reviewed. None of the previously widely accepted theories fully explains the genetics in all reported cases. It is certain from study of reported cases and their families that transmission as a simple Mendelian dominant is an impossible explanation in many instances. It is suggested that the homozygous-heterozygous theory is probably correct, but that failure of one or both parents of a sickle cell anemia patient to show sickling is the result of incomplete penetrance of the trait-producing gene. This explanation does not necessitate the assumption that much data previously accumulated are based on incorrect laboratory procedures.

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SUBACUTE ERYTHREMIC MYELOSIS*

Report of a Case in a Filipino†

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Erythremic myelosis is a rare disease. Its occurrence in the Philippines is here recorded for the first time. The report follows:

Case Report. E.L., a 30 year old, married, Filipino driver, was referred to us by Dr. Leonides Almarines of Biñan, Laguna, on May 16, 1949. The patient became aware of his illness 3 months before, when he tired easily on exertion. Palpitation and weakness followed. There was no spontaneous bleeding, no attack of precordial pain or dizziness. A few days before consultation, his blood was examined and was diagnosed as indicating aplastic anemia. Bone marrow biopsy was not done.

He stated that more than 3 years ago he contracted malaria, for which he was given atabrine. He could not remember the entire amount of the drug ingested. During the treatment he became yellow in the skin and anorexic, without nausea and vomiting. Cerebral or urinary symptoms were absent.

Physical examination on the day of consultation showed marked pallor and slight jaundice. The lower border of the liver extended 2 fingers' breadth beneath the costal arch. The spleen could not be palpated. The apex beat was diffused and cardiac dullness enlarged. A systolic murmur was heard over the pulmonary, aortic and mitral areas. There was tachycardia. Abnormal findings in other systems were absent.

Blood and bone marrow specimens were procured. The results, together with those of 2 subsequent examinations, are detailed in Table 1. Oral "Folvite" Lederle and "Reticulogen" Lilly were prescribed. On May 23, examination of the blood showed no improvement. The serum gave an icterus index of 10.5 units and an indirect Van den Bergh reaction. Amino acids and vitamin B complex were added to the original treatment. On June 5, the reticulocytes were only 4.2%. Because of the lack of improvement, hospitalization was advised. He entered the Santo Tomas University Hospital with the original complaints, which were aggravated by edema in the eyelids and in the upper and lower extremities.

He stayed in the hospital from June 13 to July 22, 1949. The lowest values for hemoglobin and erythrocytes observed were 3.7 gm. per 100 ml. and 860,000 per c.mm., respectively; the highest 7.5 gm. and 2,330,000 per c.mm. after transfusion of 1550 ml. of fresh homologous blood. The icterus index rose to 13 units on June 13 and dropped to 2.7 units on July 15. The red cell fragility was normal. Malarial parasites and malarial pigment-bearing reticulum cells were sought several times in the blood and bone marrow smears without success. Coagulation time was normal throughout. The longest bleeding time observed was 6 minutes. Cold and autoagglutinins were absent. The Widal test performed twice showed positive agglutination with Typhoid H up to 1/640 dilution. Hemoculture done twice was sterile. Weil-Felix reaction was negative. Standard Kahn test was negative. Total proteins were 6.7 gm. per 100 ml. of blood with an albumin-globulin ratio of 1.5 to 1. The urine routinely examined several times gave no abnormal findings. The stools showed 1+ eggs of ascaris lumbricoides and trichocephalus. The radiologist reported clear lungs and slightly enlarged splenic shadow. The heart traced a normal electrocardiogram. Basal metabolism was +28. Adrenalin test (June 27) for nucleated cells, performed in accordance with the method

* This is a name coined by di Guglielmo (see Leitner[6]) for the acute form of a hematologic condition that in the author's opinion has significant differences from Cooley's anemia, erythroblastosis, panhemacytopenia, and other similar diseases. EDITOR.

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TABLE 1.—HEMATOLOGIC DATA IN CASE E.L.

Peripheral Blood	5/16/49	6/25/49	7/22/49
Hemoglobin (gm.)	5.8	7.5	5.0
Erythrocytes (millions)	1.3	2.3	1.7
Vol. p.r. cells (%)	15	21	—
Reticulocytes (%)	3.2	2.0	0.5
Total WBC (including erythroblasts)	4,100	4,450	5,250
Differential count (%):			
Myeloblasts	0	0	3
Progranulocytes	0	0	2
Eosinophils	0	2	0
Neutrophilic			
Metamyelocytes	0	0	1
Band forms	1	2	2
Segmented forms	24	10	23
Erythrocytic			
Rubriblasts	2	0	0
Prorubricytes	3	2	3
Rubricytes	4	9	9
Metarubricytes	22	36	20
Mitotic RBC	0	1	0
Lymphocytes	44	38	37
Monocytes	0	0	0
Thrombocytes	109,560	18,640	58,760
Bone Marrow	hypercellular	hypercellular	hypercellular
Diff. Count (%)			
Myeloblasts	1.6	0.4	2.4
Progranulocytes	3.4	1.0	2.8
Eosinophils	present	present	0.8
Neutrophilic			
Early myelocytes	0.2	0	0
Late myelocytes	3.2	0.6	5.0
Metamyelocytes	5.6	2.4	4.8
Band	2.4	1.6	3.4
Segmented	2.4	2.2	0.8
	18.8	8.2	20.0
Erythrocytic			
Rubriblasts	1.0	1.6	4.0
Prorubricytes	4.4	3.0	10.2
Rubricytes	13.6	25.6	27.8
Metarubricytes	49.6	55.0	29.2
Mitotic RBC	1.4	1.0	1.4
	70.0	86.2	72.6
Lymphocytes	10.8	5.4	6.6
Plasmacytes	0	0.2	0
Reticulum cells	0.4	0	0.8
Myeloid-erythroid ratio	0.3	0.09	0.3
Megakaryocytes[5]	normocellular	normocellular	normocellular
Diff. Count (%)			
Amitotic form A	1	4	1
Mitotic form A	0	0	1
Megakaryoblasts	A 4, B 1	5	4 / 0	4	4 / 0	4
Promegakaryocytes	A 10, B 11	21	5 / 3	8	21 / 6	27
Megakaryocytes	A 35, B 37, C 1	73	60 / 23 / 0	83	46 / 21 / 0	67
Asynchronic megakaryocytes	0		1		0	

described by Doan and Wright[2], gave the following results:

myeloblasts from	0	to 500.25	per c.mm.
myelocytes "	76.1 "	0	"
segmented forms "	1293.7 "	1834.25	"
lymphocytes "	2891.8 "	5836.25	"
monocytes "	76.1 "	0	"
rubricytes and metarubricytes "	3272.3 "	8504.25	"
Total number "	7610.0 "	16675.00	"

Two days later the same test performed for hemoglobin and erythrocytes showed practically no increase.

The patient was ambulatory. The temperature was normal throughout, except on 2 occasions: 1, after the first blood transfusion when it rose to 38.5° C. (102.02° F.); 2, after the first injection of pentnucleotide (10 ml.). Each time it dropped to normal the following day. In view of the temperature curve, the positive Widal test was taken to be a pure coincidence (patient has no history of previous vaccination against typhoid) without any bearing on the hematologic disorder. Edema of the eyelids and lower extremities appeared when the red cell count fell to less than 1,500,000 per c.mm., and disappeared after whole blood transfusion.

The medicines prescribed were Chlorguanide, Folvite, multivitamins and amino acids. When they failed to produce any improvement, pentnucleotide was tried. After the fifth injection, the patient asked for his discharge.

When he left, the jaundice and edema were absent. While he declared that he was feeling a little stronger than when admitted, the bone marrow findings (Table 1, 7/22/49) indicated that his condition had become worse. Nothing was heard of him till a few weeks later, when he died in his home. No details of his last days could be secured. Autopsy was not performed.

Cytological Description. The blood and bone marrow smears were fixed with Wright's solution and stained with a combination of May-Grünwald's and Giemsa's. The platelet counts were determined according to the wet-smear method of Dameshek.

The terms, recommended by the Committee for Hematologic Nomenclature of the American Society of Clinical Pathologists[7], were adhered to as much as possible. *Erythroblast* is used in the collective sense to denote any nucleated red cell. The megakaryocytes are classified according to the system we had proposed before[5].

All the photomicrographs were taken at the same magnification. The size of any cell may be calculated from the cell in Fig. 2, whose longest diameter measures 37.8μ.

The bone marrow was hypercellular throughout.

Erythrocytic Series. In the 3 biopsies made, 70%, 86.2% and 72.6% of the total marrow nucleated cells belonged to this series. Undoubtedly the hypercellularity of the bone

marrow was due to an excessive proliferation of the erythroblasts. None of the latter was of the pernicious anemia type. Of the total nucleated red cells, metarubricytes constituted 71% in the first examination, 64% in the second and only 40% in the third. This indicated that while at the time of the first biopsy disturbance in maturation was not perceptible, the succeeding examinations disclosed a definite progressive arrest in the ripening of cells.

The rubriblasts and prorubricytes were larger than ordinarily observed (Fig. 1). Occasional multinucleated giant cells (Figs. 2 and 8) were present. Tri-nucleated and binucleated figures were numerous. An outstanding peculiarity was the asynchronic development of the twin nuclei of a cell. The binucleated cells in Fig. 4 illustrate this phenomenon. In each cell, one nucleus is more mature than its twin. In Fig. 5, the big (right) nucleus is at the prorubricytic stage, while the small one is in prophase of mitosis.

The latter is indeed a very rare developmental anomaly. Berman[1] and Rebuck[8] in their investigation on cellular gigantism and multinuclearity did not touch on it. Due to the unavailability of references, published before the last war, we are not in a position to state whether such disorder had already been described before. Certainly, it indicates a severe disturbance in cellular growth. In Figs. 4, 5 and 8, anisonucleosis is also apparent. All these findings would incline one to consider erythremic myelosis, or at least this case, as a neoplasm of the erythropoietic tissue.

The condition of the bone marrow was reflected in the peripheral blood as a severe anemia with erythroblastosis. The counts of nucleated red cells per c.mm. in the 3 examinations recorded in Table 1 were 1271, 2136 and 1690 respectively. There were more erythroblasts than granulocytes circulating at any time. During the adrenalin test, the increase of the former was greater than that of any single type of white cells. Howell-Jolly bodies abounded. Anisocytosis was marked. Hypochromia of a few cells was observed. Poikilocytosis was slight.

The reticulocytes decreased progressively with the arrest in maturation of the erythroblasts. No satisfactory explanation for the transient jaundice could be found.

Granulocytic Series. The granulopoietic tissue was hypoplastic, since the time of the first biopsy. Of the total nucleated marrow cells, only 18.8% belonged to the series, while

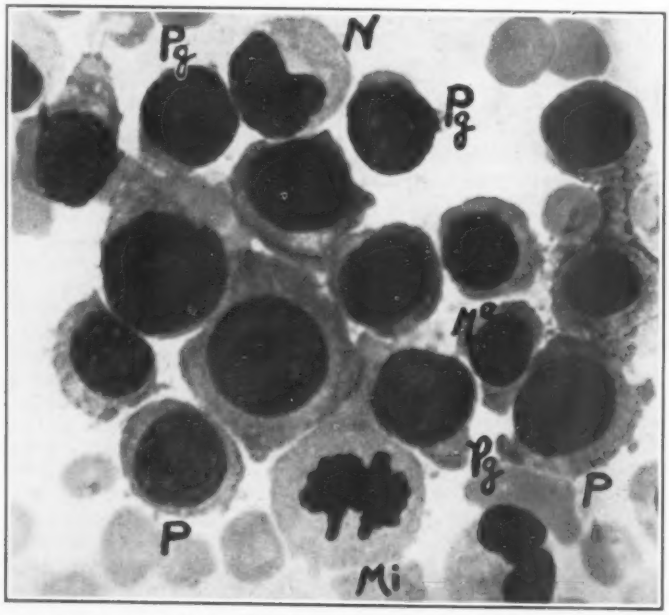


FIG. 1.—Bone marrow. The 2 biggest cells are rubriblasts; P, Prorubricytes; Pg, Progranulocytes with small cytoplasm containing few, tiny, barely visible granules; N, Neutrophile metamyelocyte; Mi, Mitotic erythroblast. The rest are rubricytes. Me, a rubricyte having the same nuclear structure as the cell in Fig. 8.

in the peripheral blood the granulocytes numbered only 1,025 per c.mm. In subsequent examinations, the count oscillated with wide amplitude, but at no time did it reach the normal value. At the peak of the splenic contraction (coincident with the highest point of systolic blood pressure) during the adrenalin test, the granulocytes (myeloblasts and neutrophils) in the peripheral circulation rose to 2334.5 per c.mm., a figure below the minimum normal for this cell type.

Morphologic abnormalities were also present. Granules were lacking in the cytoplasm of many cells (Figs. 6 and 7). It appeared as if the cytoplasm was devoid of content and consisted merely of membrane. The nucleus was also affected. Normally, as the cell matures and the nucleus assumes a lobulated form, the chromatin materials clump into coarser strands. In the present case, the delay in clumping was very noticeable. Leitner[6] has seen the same phenomenon in pernicious anemia and called it the "dissociation of maturation in nuclear shape and structure". In the present case, this asynchronism is especially true in the myelocytic and metamyelocytic stages (Figs. 6 and 7). Hence, in the differential count, the cell classification was based solely on the shape of the nucleus. Nuclear hypersegmentation, a characteristic finding in pernicious anemia, was not observed in the present case. The higher values in the last examination (7/22/49) in both bone marrow and peripheral blood might have been due to the pentnucleotide.

Thrombocytic Series. The total number of megakaryocytes was normal in all 3 examinations. Only in the first biopsy were "C" cells[5] (megakaryocytes containing more than 10 platelets in the cytoplasm) present. Even the "B" cells (containing less than 10 platelets) were very much diminished in succeeding examinations.

An unusual finding was the phagocytosis of erythroblasts by the promegakaryocytes (Fig. 3) and megakaryocytes. In many bone marrows, normal as well as pathological, one may detect a rare mature megakaryocyte engulfing a leukocyte or an erythroblast. But in the present case, the phagocytic megakaryocytes were conspicuous by their number. Another abnormality was the tendency of the nucleus to hypersegmentation, similar to but not as exaggerated as that observed in pernicious anemia[3].

There was thrombocytopenia (see Table 1) throughout the entire course of the disease. Large thrombocytes were present, as they are in many conditions where these elements are low in number. However, the longest bleeding time (Duke) observed was 6 minutes. This occurred only once. At other times it was within normal limits. Coagulation time (Lee-White) was normal. There was no spontaneous bleeding before and during hospitalization.

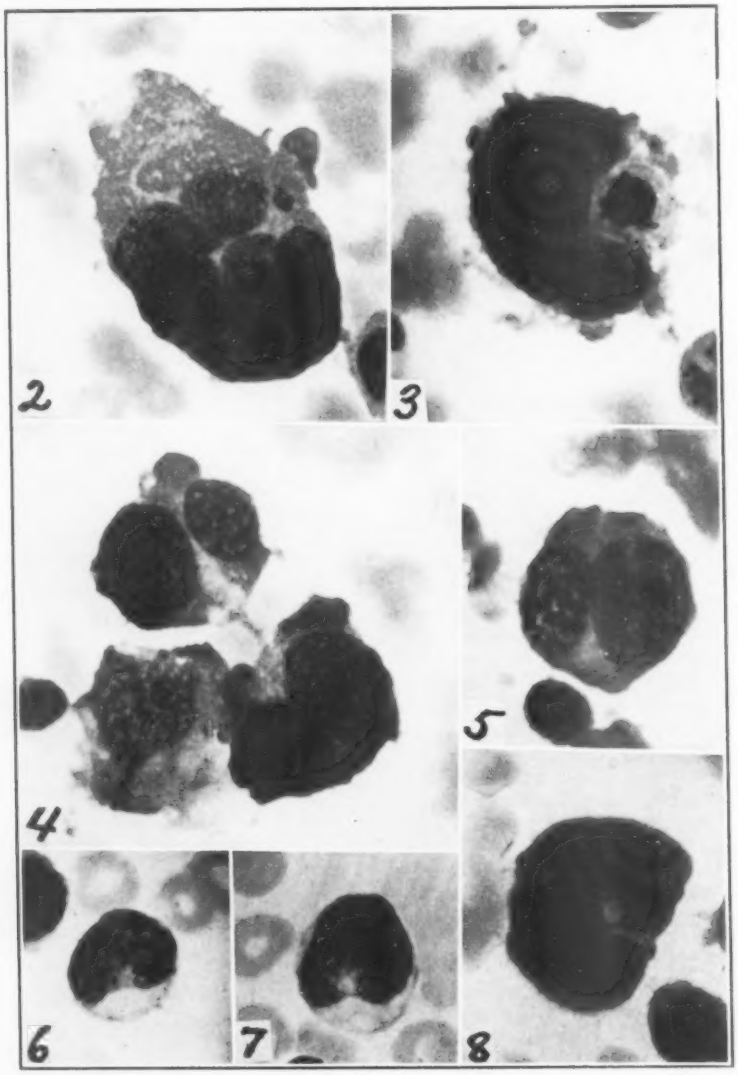
No abnormality was found in the lymphocytes, monocytes and reticulum cells.

Discussion. Briefly, the present case was characterized by: 1, a course of about 4 months, hepatomegaly, slight splenomegaly and transient jaundice; 2, pancytopenia and erythroblastosis in the peripheral blood; and 3, excessive proliferation of the nucleated red cells with arrest of maturation, hypoplasia and disturbed development of the granulocytic series, deficient thrombocyte formation and excessive phagocytosis of the megakaryocytes, with concomitant morphological alterations in these 3 cellular elements of the bone marrow. These findings would satisfy the criteria for the diagnosis of subacute erythremic myelosis[6].

The unavailability to us of the original articles on the subject makes it impossible to compare our case with those reported by earlier investigators. Compared with Leitner's[6] summary of the literature on this hematologic disorder, it seems that previous writers had stressed more the pathology of the erythropoietic tissue with but scant mention of the granulocytic and thrombocytic cells.

All 3 of the principal cellular constituents of the bone marrow were affected. However, if number and severity of the lesions were the basis for establishing the primary seat of injury, the changes in the erythropoietic tissue would be accepted as the fundamental disturbance.

The cause of erythremic myelosis is still disputed. The kind of lesion to us suggests neoplasm as the nature of the disorder. Our patient had malaria, treated with atabrine. However, any causal relation between malaria or ata-



FIGS. 2 to 8.—Bone marrow. 2, Giant multinucleated prorubricyte, whose longest diameter measures 37.8μ. 3, Promegakaryocyte B with engulfed metarubricyte. 4, Two binucleated erythroblasts with basophilic cytoplasm. The nuclei in each cell are at different phases of development. The difference in size of the twin nuclei of the upper cell is apparent. The third cell (lower right) is an eosinophilic myelocyte. 5, Binucleated erythroblast. One nucleus (right) is bigger and at the prorubricytic stage, the other in prophase of mitosis. 6 and 7, Neutrophilic metamyelocytes, illustrating the dissociation of maturation in nuclear shape and structure and the absence of granules. 8, Giant multinucleated rubricyte. The cytoplasm is homogeneously deep greyish-blue, except at a small circular area near the center.

brine and the hematologic disorder under study is too slight to be considered seriously, at least for the present. The effects of atabrine intoxication in the blood, as shown by previous investigators[4], are entirely different. observations on malaria bear no similarity with the present case.

Summary. A case of subacute erythremic myelosis found in a Filipino is reported. The hematologic disorder involves the 3 principal cellular types of the blood and their precursors. The cytological abnormalities are described in detail and the cause and nature of the disease briefly discussed.

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LEUKEMIA IN RADIOLOGISTS IN A 20 YEAR PERIOD

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Some experimental evidence has been presented to show that exposure to ionizing high energy irradiation (by Roentgen irradiation) can produce leukemia. Heineke[10], Ziegler[21], and Fischer-Wasels[3] reported that the general irradiation of mice with Roentgen rays caused a myeloid transformation of the tissues of the spleen. Calò[1] observed the appearance of large histocytic infiltrations in the organs of white mice which had been exposed to small doses of Roentgen rays. Krebs, Rask-Nielson, and Wagner[16] found that among 10,500 normal mice there occurred 6 cases of the leukemic state (0.058%), while among 5550 subjected to Roentgen rays, there were 19 cases (0.36%). These authors do not attempt to distinguish between a true leukemia with peripheral blood changes, an aleukemic lymphadenosis, a lymphosarcoma, or a leukosarcomatosis, because of the various gradations that may exist between these different forms. Furth, Seibold, and Rathbone[8,9] found that most, if not all, resistant strains of mice can be made susceptible to transmissible leukemia through exposures to single or repeated doses of Roentgen rays. Furth[4] further showed that myeloid leukemia rarely occurred in his mice unless they were irradiated. Of 481 controls, 2 died of myeloid leukemia; of 385 irradiated mice, 21 died with myeloid leukemia, a 13-fold increase. In a subsequent study, Furth and Furth[7], using 1290 controls and 775 irradiated mice, found that irradiation produced 8 times the incidence of myelosis (6.8% as against 0.9%). In the majority of cases there was a conspicuous invasion of the circulating blood by immature myeloid cells (leukemia). Generalized lymphomatosis with or without blood involvement likewise occurred more often after irradiation in all of the 3 stocks that they used, but this increase was only 2-fold.

Hueper[13] found that total irradiation of a highly susceptible strain of cancer mice with small doses of Roentgen rays resulted in a marked increase in the incidence of leukemia that normally occurred (74% as against 19%) in animals of the strain used. He thought that hyperplastic myeloid or lymphoid proliferations were transformed into leukemic ones by the effect of Roentgen rays upon the regenerative activity of these tissues. Henshaw[11] gave repeated moderate single doses at 4 week intervals and found more than a 4-fold increase in incidence of leukemia (in its broader sense) in a moderately susceptible strain of mice. Furth and Boon[6] found that the leukemogenic action of methylcholanthrene was greatly enhanced by preirradiation with doses of Roentgen rays (175 r), which alone rarely produces leukemia, and the onset of leukemia occurred in a shorter interval. Kirschbaum and Kaplan[15] point out that the problem of leukemogenesis in mice is very complex: 1, multiple agents (Roentgen rays, carcinogens and other chemical agents, estrogens) can produce leukemia; 2, mice of only certain genetic constitution are sus-

ceptible to only certain agents; 3, genetic susceptibility to one agent, or to the spontaneous disease, cannot necessarily be correlated with susceptibility to other leukemogenic agents. Furth[5], in a comprehensive review, states that from a survey of the literature it appears that Roentgen rays are weak leukemogens (relative to other experimental agents) and act after a relatively long incubation period. In all of the above experimental procedures, the Roentgen rays initially exert a destructive influence on the leukopoietic foci, following which there is a period of regeneration in which frequent mitotic figures are seen and abnormal forms are produced. Persistence and multiplication of the latter produces the leukemic state.

In a more recent study, Lorenz *et al.*[17] have investigated the effect of prolonged low-dosage irradiation on mice, guinea pigs, and rabbits in an attempt to determine tolerance ranges. They found in mice that the time in which leukemia (lymphoma) appears is approximately the same for those animals receiving up to 2.2 r per 8 hours per day as in the controls, and the percentage incidence was also the same. With somewhat larger doses there was an earlier onset of leukemia, and with 8.8 r per 8 hours per day not only was the onset much earlier but there was a 70% incidence instead of a 45% incidence. The authors prefer to explain this higher incidence on the basis of the earlier onset, thinking that otherwise susceptible mice in the less irradiated group might have died of other causes before the onset of leukemia. Exception might be taken to this interpretation. The results in guinea pigs and rabbits are not given, but in a subsequent paper Lorenz[18] states that no increase of leukemia incidence was observed in the guinea pigs or rabbits. He notes, however, that the number of animals used in these latter experiments is small. From his experiments Lorenz[18] concludes that "in spite of the statistical evidence (of the increased incidence of leukemia in radiologists) reported, the data obtained in (my and my associates') experimental animals do not seem to support the view that chronic irradiation (of the amounts to which I believe radiologists are exposed) will induce leukemia in man unless one is willing to assume differences in the susceptibility and the mechanism of leukemia induction in humans." That such an assumption may not be entirely out of order is indicated by the known differences in susceptibility found in different strains of mice, and the species difference in susceptibility demonstrated in the varying results obtained in mice, guinea pigs and rabbits in these experiments.

In a companion study to the preceding, on the same animals, Jacobson and Marks[14] studied the peripheral blood findings in these animals and found no peripheral lymphocytosis occurring in any of the 3 species. A lymphopenia as the result of chronic low-grade irradiation was the consistent finding and the earliest abnormality noted.

What might be regarded as a form of clinical corroboration of the relationship between Roentgen ray exposure and increased incidence of leukemia in humans was presented several years ago when this writer[19] pointed out that the incidence of leukemia (in its narrower sense) was more than 10 times as great in radiologists as in non-radiological physicians. This difference was shown to be statistically significant. The present report is a continuation of the previous study and includes the ensuing 5 year period, 1944 to 1948, inclusive. The same method was followed as in the previous paper, extreme care being

taken to obtain as complete figures as possible and to avoid any duplication or the inclusion of any non-pertinent subjects. For a more detailed discussion of the method, reference can be made to the previous paper. It is probable that a very few of those listed as radiologists (but none of the leukemia cases) were in reality surgeons or other specialists who were members of one of the national radiological societies. This factor would tend to dilute slightly the actual incidence rate for bona fide radiologists.

During this 5 year period, the deaths of 15,762 United States physicians were reported in the Journal of the American Medical Association. During this same period, the Directory Report Service of the American Medical Association listed 19,529 deaths, the discrepancy resulting from the fact that the Journal does not publish an obituary for every physician who dies. Since, however, our present interest is in causes of death, the Journal's figures are being used. Leukemia was reported as the cause of death in 119 of these. During the same period, 124 radiologists died; in this group (all of whom had the cause of death recorded) 6 were reported as dying of leukemia, as indicated in Table 1.

In Table 1 might have been included Dr. W. B. C., who was not strictly a radiologist but a renowned physiologist. However, he was a member of the American Roentgen Ray Society and had used Roentgen rays and fluoroscopy very extensively in physiological research from the earlier days of these modalities. He developed leukemia cutis (mycosis fungoides) for which he received Roentgen therapy over a period of 15 years. In the obituary notice appearing in the Journal of the American Medical Association, he is reported to have died of leukemia, but in the one appearing in the American Journal of Roentgenology and Radium Therapy, the cause of death is said to have been a malignant lymphoma, apparently from more positive information. Since it has been the policy in this study to include only positive leukemia cases, this case was excluded. Likewise, no other case of lymphosarcoma was included.

TABLE 1.—RADIOLOGISTS DYING OF LEUKEMIA

Initials	*Date of Death*	*Type of Leukemia Recorded*	*Age*	*Location*
N. J. N.	July 2, 1948	Acute	67	Sioux Falls, S. D.
L. G. A.	May 28, 1948	Myelogenous	57	Kansas City, Ka.
R. H. S.	May 16, 1946	Acute	78	Detroit, Mich.
E. R. B.	June 16, 1946	Chronic Myelogenous	58	Cincinnati, Ohio
C. W.	May 29, 1945	"Leukemia"	68	Boston, Mass.
A. W. J.	Aug. 8, 1945	"Leukemia"	52	New York, N. Y.

It is seen from these figures that the incidence of leukemia in non-radiological physicians for this 5-year period is 113 in 15,637 (0.72%). The incidence of leukemia in radiologists during this period is 6 in 124 (4.84%). These percentages approximate those reported for the preceding 15-year period in both non-radiological physicians (0.44%) and radiologists (4.57%). This serves as some additional confirmation of the validity of the claim that the figures previously presented were statistically significant. The combined figures for the 2 decades 1929 to 1948 inclusive become: 65,922 non-radiological physicians died, of whom 334 died of leukemia. During this same 20-year period, 299 radiologists died, of whom 14 had leukemia. The over-

all incidence for non-radiological physicians is 0.51% and for radiologists 4.68%.

It is thus seen that the incidence of leukemia in radiologists is over 9 times as great as in non-radiological physicians. This order of increase is larger than was obtained in most of the experimental work on mice. In spite of the relatively low incidence rate in man, both groups under consideration, the non-radiological physicians and the radiologists, are now sufficiently large and the statistics cover a sufficient number of years for this difference in incidence rate to bear the stamp of validity. Moreover, by applying the same statistical analytical evaluation as in the previous paper (expansion of the binomial $(p + q)^n$ to the appropriate term) it is found that this term equals 1×10^{-9}, indicating that there is only 1 chance in a billion of the observed increased incidence rate of leukemia in radiologists being coincidental.

It has been observed[2,12] that the incidence of leukemia is almost twice as great in physicians as a whole as in the general adult male white population. The writer[20] has pointed out that the bulk of this increase was due to the inclusion of radiologists in the statistics for physicians, and that when only non-radiological physicians were considered, the increased incidence is only slight and might perhaps be accounted for by the rather considerable number of non-radiological physicians who use fluoroscopes, Roentgen-ray machines, radium, and now radioactive isotopes in their clinical or investigative work.

This occupational hazard of radiology indicates that: 1, the hazard was not sufficiently appreciated, and insufficient care was exercised to employ standard means of protection against ionizing radiation; or 2, these standard means of protection are insufficient protection; or 3, they are too difficult to employ correctly in routine work. In this connection, the work of Archer at the University of Virginia is interesting. He has lately developed and exhibited a gown made of spun lead glass that affords much more complete protection to the body than conventional lead apron and gloves.

Summary. 1. The experimental work showing an increased incidence of leukemia in mice exposed to Roentgen irradiation has been reviewed.

2. The study of the incidence of leukemia in radiologists has been extended to cover a 20 year period.

3. It has been shown that during this time leukemia has occurred more than 9 times as frequently in radiologists as in non-radiological physicians.

4. This difference has statistical significance.

5. It is suggested that the increased incidence of leukemia in physicians as a whole (compared with the adult male white population) might be due to the same factor of exposure to ionizing radiation.

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ADRENAL CORTICAL FUNCTION IN ESSENTIAL HYPERTENSION
A STUDY OF SWEAT SODIUM CONCENTRATION

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Adrenal cortical hypersecretion as seen in Cushing's syndrome or after large doses of ACTH is associated with an elevation in arterial blood pressure. This hypertension often regresses after the hypersecreting adrenal cortical tissue has been surgically excised, thus indicating a causal relationship. Hypertension can also be produced readily in several mammalian species by prolonged overdosage with desoxycorticosterone acetate (DCA), even though adrenal cortical extract will not usually cause it[4,5].

The hypertension associated with Cushing's syndrome is accompanied by retinopathy, A-V nicking, benign and malignant nephrosclerosis, left ventricular hypertrophy, cardiac failure, increased susceptibility to cerebral vascular accidents and myocardial infarctions exactly as seen in essential hypertension. Even the microscopic changes in the arterioles are similar in the two conditions.

Furthermore, the adrenal cortex seems necessary to maintain the increased arterial blood pressure associated with essential hypertension, since this elevated blood pressure will revert to normal levels if adrenal cortical insufficiency supervenes. This drop in blood pressure will occur even though the blood volume and extracellular fluid volume are at normal levels as a result of massive NaCl ingestion[3].

With these data in mind, it seemed important to evaluate the level of adrenal cortex function in essential hypertension. It is especially necessary to explore the adrenal cortical steroids of the "electrolyte influencing" type, such as desoxycorticosterone acetate (DCA), since they are especially potent in producing hypertension.

Conn *et al.* showed that this type of steroid had a striking effect on the sodium and chloride concentration in thermal sweat. Large doses of ACTH or DCA caused a fall in sweat sodium concentration to the low levels seen in patients with Cushing's syndrome. Conversely, patients with Addison's disease had abnormally high sweat sodium concentrations which decreased to the normal range after DCA therapy[1]. The sweat sodium concentration is probably the best index of the activity of "electrolyte-influencing" adrenal cortical steroids.

Methods. The entire study was completed during the winter and spring. The subjects were placed in a room at 99° F. with 97%

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relative humidity for 25 minutes. During the last 10 minutes of this period, sweat was scooped off the back with test tubes. Sweat was analyzed for sodium by the method of Hoffman and Osgood[2]. All hypertensive patients with anemia or congestive heart failure were excluded. Patients were not on any diet involving salt restriction.

TABLE 1.—33 NORMOTENSIVE SUBJECTS

Blood Pressure mm. Hg	*Sweat Sodium Concentration m.Eq./liter*
130/80	40.9
106/70	25.4
138/86	43.9
104/64	42.2
142/72	30.0
136/88	86.6
134/84	65.7
148/76	53.1
124/80	25.2
132/78	19.8
110/72	61.8
118/68	43.0
126/72	71.4
112/64	32.6
130/76	37.4
120/68	33.7
126/76	24.6
108/70	43.9
136/82	11.5
114/66	54.2
128/72	28.2
148/78	55.6
104/76	23.5
130/66	29.6
130/76	49.6
130/80	6.5
120/76	40.4
112/70	19.3
132/66	52.6
100/65	34.8
130/78	27.4
104/70	5.4
120/68	39.6
Mean: 124/74	Mean ± S.E.M.: 38.2 ± 3.1

Results. In Table 1 are shown the individual values of sweat sodium concentration for 33 normotensive subjects. The individual values for 35 hypertensive subjects are shown in Table 2. All these hypertensives had a diastolic pressure of at least 100 mm. Hg.

In Table 3 the mean sweat sodium concentration is shown for the 11 hypertensives with more than 125 mm. Hg. diastolic pressure.

In Table 4 the average sweat sodium concentration for the 22 hypertensives with a diastolic pressure between 100 and 124 mm. Hg. is shown.

Statistical analysis shows that there is no significant difference between any of these groups.

TABLE 2.—35 HYPERTENSIVE SUBJECTS

Blood Pressure mm. Hg	*Sweat Sodium Concentration m.Eq./liter*
230/170	24.6
210/156	13.9
250/150	17.4
230/140	31.3
246/138	90.5
258/134	37.4
200/130	12.2
240/130	35.6
250/128	20.0
220/126	74.6
170/126	13.5
218/124	61.6
234/124	70.0
230/124	41.5
196/122	25.2
230/120	50.4
190/120	38.2
220/120	42.2
224/120	74.2
194/120	13.7
208/120	37.8
180/118	19.1
170/118	59.2
180/118	23.0
220/112	56.1
200/110	62.4
180/110	17.8
180/110	30.0
180/108	60.4
180/108	101.3
150/108	101.3
160/106	15.4
166/106	52.8
150/100	41.9
170/100	72.6
Mean: 203/122	Mean ± S.E.M.: 44.0 ± 4.4

TABLE 3.—11 HYPERTENSIVES WITH DIASTOLIC PRESSURES OVER 125 MM. HG

Average Blood Pressure	*Mean Sweat Sodium Concentration ± S.E.M. m.Eq./liter*
228/139	33.7 ± 7.9

TABLE 4.—24 HYPERTENSIVES WITH A DIASTOLIC BLOOD PRESSURE BETWEEN 100 AND 124 MM. HG

Average Blood Pressure	*Mean Sweat Sodium Concentration ± S.E.M m.Eq./liter*
192/114	49.5 ± 5.2

Comment. In view of these results, it is quite unlikely that the ordinary patient with essential hypertension has any considerable increase in the level of "electrolyte influencing" adrenal cortical steroids.

It is still possible that there might be a slightly increased secretion in hypertensives, which is too small to effect a lowering of sweat sodium concentration.

It is also possible that in hypertensives normal amounts of adrenal cortical steroid are secreted but then enter abnormal metabolic pathways with the creation of abnormal metabolites which influence blood pressure but not sweat sodium.

Summary. 1. The sweat sodium concentration in patients with mild or severe essential hypertension is not significantly different from that of normotensive subjects.

2. This makes it very unlikely that ordinary essential hypertensives have any considerable increase in the level of "electrolyte-influencing" adrenal cortical steroids.

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EXPERIENCE WITH PROTEIN BOUND IODINE (PBI); THE EFFECT OF ACTH AND CORTISONE ON THYROID FUNCTION

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Many investigators have studied the nature and levels of the PBI of serum[1,2,3,4,6,11,12]. There is general agreement that this iodine fraction represents thyroxine and its immediate precursors, and that it is a reliable measure of the functional activity of the thyroid gland. Using radioactive iodine, Taurog and Chaikoff[13] showed that of the precipitable iodine almost 90% could be crystallized and was identical with thyroxine by all physical and chemical methods available. The purpose of the present study was to apply an accurate method of PBI determination to the investigation of states of abnormal endocrine activity.

Methods and Materials. The permanganate method of Riggs and Man[6,10] was found to be satisfactory for the measurement of serum precipitable iodine. Normal values reported by these workers ranged from 3.5 to 7.0 micrograms (γ) per 100 ml. of serum. As with other methods, care must be exercised that the patient has received no iodine compound for an appropriate length of time preceding the examination. Man and Peters[5] have shown that priodax and diodrast may cause an elevation of the serum PBI for as long as 16 weeks; lipiodol instilled for bronchography may give spuriously elevated PBI levels up to 15 months.

Two groups of subjects were studied: normal hospital personnel and patients with collagen diseases treated with ACTH and cortisone. In the last group the serum PBI levels were determined immediately preceding and immediately following the course of therapy. In general, 100 mg. of ACTH or cortisone were given daily in divided doses for from 7 to 10 days.

Results. The PBI values obtained in our normal subjects ranged from 3.0 to 6.0 micrograms (γ) per 100 ml. of serum and thus were in substantial agreement with those of Riggs and Man[6,11].

The serum PBI values obtained before and after treatment with ACTH or cortisone are presented in Table 1. Where both pre-treatment and post-treatment serum levels were obtained, the PBI levels diminished during therapy with ACTH or cortisone in all patients. In those patients where no pre-treatment blood samples could be obtained the serum PBI levels subsequent to treatment were in all instances below the normal level.

Discussion. While we have found no data such as these in the literature, Sprague and associates[9] at the Mayo Clinic have recently reported a moderate decrease in the BMR following both ACTH and cortisone therapy. This did not occur following the administration of Compound F. The exact mechanism of this finding was not clear, but the possibility that it was due to a general decrease in metabolic

activity independent of altered thyroid function was not excluded. Paschkis, Cantarow, Eberhard and Boyle[7] have shown that the alarm reaction which follows the injection of formalin into guinea pigs is accompanied by a decreased uptake of radioactive iodine by the thyroid gland. Soffer, Gabrilove and Jailer[8] found that the injection of epinephrine decreased radioactive iodine uptake in normal animals, but increased it in adrenalectomized animals. This increased uptake was prevented by the administration of cortisone. The authors concluded that the adrenal cortex exerts an inhibitory effect on the thyrotropin stimulating effect of epinephrine. It is possible that this was the mode of action of ACTH and cortisone in our patients. Whatever the exact mechanism involved, it is important to bear in mind that the effects of ACTH and cortisone are widespread and that the beneficial effects resulting from the administration of these compounds may be mediated through multiple metabolic processes.

We are now studying the effect of ACTH and cortisone on the serum PBI level in hyperthyroid states.

TABLE 1.—EFFECT OF ACTH AND CORTISONE ON THE SERUM PBI LEVELS.*

Patient	*Diagnosis*	*Drug*	*Before*	*After*
I. C.	Arthritis	Cortisone	5.3	1.7
J. K.	Uveitis	Cortisone	4.2	1.1
D. W.	Arthritis	Cortisone	—	3.2 (after 2 days treatment)
			—	1.3 (after 10 days)
M. H.	Lupus Erythematosus	ACTH	—	1.9
J. R.	Leukemia	ACTH	—	1.7
E. B.	Lupus Erythematosus	ACTH	4.9	2.3
M. K.	Lupus Erythematosus	ACTH	4.9	2.1
M. C.	Arthritis	ACTH	2.3	1.3
H. H.	Arthritis	ACTH	4.7	1.5
R. G.	Arthritis	ACTH	5.8	2.7
J. W.	Uveitis	ACTH	11.0	8.0
H. W.	Exophthalmos	ACTH	6.2	2.8

* The PBI values before and after treatment are expressed as per 100 ml. of serum.

Summary and Conclusions. 1. The serum protein bound iodine (PBI) levels have been studied in normal subjects and in patients undergoing ACTH or cortisone therapy for collagen diseases.

2. The administration of ACTH or cortisone to patients with various of the collagen diseases resulted in a decrease in the serum PBI in all patients. The mechanism by which this effect may be mediated is discussed.

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MILIARY TUBERCULOSIS OF THE LIVER: LIVER BIOPSY AS AN ADJUNCT TO DIAGNOSIS*

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Miliary tuberculosis of the liver is not a commonly recognized clinical entity. While it is true that miliary tubercles may be found in the liver at autopsy in 50 to 80% of cases of pulmonary tuberculosis[5,7], the syndrome of fever, chills, and enlarged liver, with or without jaundice in the absence of discernible pulmonary involvement has been considered rare[8,11,12].

The difficulty to substantiate, or even suspect, a diagnosis of miliary tuberculosis of the liver has been appreciated by previous authors as well as ourselves. A specimen of liver tissue for histological study would be of unquestionable value. Few reports have appeared in the literature concerning the value of needle biopsy in diagnosing miliary tuberculosis of the liver[2,3].

We are presenting 3 cases of miliary tuberculosis of the liver diagnosed by liver biopsy. They are also of interest, in that idiopathic hyperpyrexia was the presenting problem in 2 of the cases. In the 3rd case, fever and jaundice were the salient features. In no case was there roentgenological or bacteriological evidence of tuberculosis prior to needle biopsy of the liver. All cases were treated with streptomycin.

Case Reports. Case 1. This 34 year old, asthenic negro male entered the hospital on November 17, 1945, complaining of weakness, weight loss, and abdominal distention of 2 weeks' duration. He had an intermittent daily fever ranging from 99 to 103°F.

Physical examination revealed the following significant findings: a lag of the right chest on inspiration with dullness and impaired breath sounds at the right base; abdominal distention due to ascites; a barely palpable liver edge below the right costal margin; and 1+ pretibial edema.

Chest Roentgen ray showed elevation of the right leaf of the diaphragm and a small amount of fluid in the right costophrenic sulcus. Blood Kahn test, complete blood count and urine were essentially normal. PPD skin test (0.00002 mg. strength) was positive. A number of stools were negative for ova, parasites and enteric pathogens. Blood non-protein nitrogen was normal. Many blood smears were negative for malaria parasites. The blood culture was sterile. Icteric index was 6. Serum proteins were 8.8 gm. per 100 cc. with an albumin/globulin ratio of 0.6/1. Bromsulfalein dye excretion showed 35% retention in 45 minutes. Blood agglutination tests for typhoid, paratyphoid and brucella were negative. Ten sputum examinations were negative for acid-fast bacilli. Urine culture was negative for tubercle bacilli.

Shortly after admission the patient required abdominal paracentesis. Studies of this fluid showed a specific gravity of 1.026 and 150 white blood cells which were predominantly lymphocytes. A culture of the fluid

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for tubercle bacilli and guinea pig inoculation was negative. The febrile course and the weight loss continued. One month after admission a repeat abdominal paracentesis yielded fluid which again was negative on guinea pig inoculation and culture for tuberculosis. Three months after admission there was clinical and roentgenographic evidence ot fluid in the right thorax although there was no parenchymal pulmonary infiltration. Thoracentesis on the right was accomplished with removal of 1800 cc. of clear amber fluid. Smear, culture and guinea pig inoculation of this fluid were negative for tuberculosis.

The patient left the hospital against medical advice after 7 months. At that time, he was still having daily temperature elevations. The cause of the fever, pleural effusion, ascites and general debilitation had not been established.

He returned to the hospital 1 year later. He had done well at home until 10 days prior to admission when he noticed the return of abdominal swelling and dyspnea.

Physical examination showed dullness and absent breath sounds over both lung bases. There was moderate ascites along with 4+ edema of the lower extremities. The liver was palpable 2 fingers' breadth below the right costal margin.

Laboratory data: blood Kahn test was negative. Urinalysis showed 2 to 4 plus albuminuria with occasional granular casts and specific gravity of 1.020. Phenolsulphonephthalein excretion was 45% in 2 hours. Blood non-protein nitrogen was normal. Red blood count showed a moderate secondary anemia. There was no leukocytosis. Serum proteins were 6.8 gm. per 100 cc., with an albumin/globulin ratio of 0.6/1. Cephalin flocculation test was negative. Icteric index was 4. Agglutination tests for typhoid, paratyphoid, brucella and tularemia were negative. Blood cholesterol was 220 mg. per 100 cc., with 77% esterification. Many malaria smears were negative. Ten sputum examinations for acid-fast bacilli were negative. Many blood cultures were negative. Congo red test was normal. Numerous stool studies were negative for enteric pathogens, ova, and parasites. Repeated urine cultures were negative for acid-fast bacilli. PPD skin test (0.00002 mg. strength) remained positive.

Roentgen ray of the chest on admission confirmed the clinical impression of bilateral hydrothorax. Thoracentesis was performed on the left with removal of 800 cc. of straw-colored fluid. This fluid was negative on culture and guinea pig inoculation for tuberculosis. Daily elevations of temperature ranging from 100 to 104°F persisted. Thoracentesis was repeated on the left, 5 days after admission, with removal of 1200 cc. of straw colored fluid. This was again negative on culture and guinea pig inoculation for acid-fast bacilli. One month after admission the peripheral edema and ascites had spontaneously mobilized. There was progressive weight loss, however, as well as a continued septic course. Two months after admission, Roentgen ray demonstrated complete clearance of the pleural effusion and there was no infiltration of the lung parenchyma present. Three months after admission there began 3 to 4 liquid bowel movements daily and dependent edema reappeared.

On December 19, 1947., a needle biopsy of the liver was performed. The pathological report was as follows: "Several portal islets are enlarged by the formation of tuberculous granulomas. These are composed of small irregular areas of necrosis surrounded by a zone of spindle-shaped fibroblasts, small lymphocytes and occasional Langhans' giant cells. Liver cells adjoining these lesions are atrophic and frequently vacuolated. Diagnosis: Miliary Tuberculosis of Liver." (Fig. 1).

The patient was started on streptomycin, receiving 2 gm. daily in divided doses. Despite this therapy there was progressive decline and he died 10 days later.

At autopsy miliary tuberculosis involving the lungs, lymph nodes, liver, spleen, adrenals, left kidney and intestines was found.

Case 2. This 54 year old white male entered the hospital on April 26, 1949, complaining of easy fatigability and a 50 lb. weight loss of 1 year's duration.

Physical examination: cachexia. Temperature 101°F; chest negative; liver palpable 2 fingers' breadth below the right costal margin; and questionable ascites.

Laboratory examinations revealed blood Kahn test, complete blood count and urine normal. Liver function tests revealed 3+ cephalin flocculation, 12% bromsulfalein retention, normal icteric index, and total proteins of 8.6 per 100 cc. with albumin/globulin ratio of 0.84/1. PPD skin test (0.00002 mg strength) was positive. Ten sputum smears were negative for acid-fast bacilli. Repeated gastric washings were negative on smear and culture for tuberculosis. Blood culture was negative. Roentgenogram of the chest showed numerous nodular infiltrations throughout both pulmonary fields considered to be pneumoconiosis. There was also infiltration in the left upper lung field interpreted as pneumonia.

A needle biopsy of the liver was performed

The pathological report was as follows: "There is an area of loose granulation tissue containing some lymphocytes, epithelioid cells, one Langhans' type giant cell and small strands of acidophilic homogeneous material. Lymphocytes are increased in the periportal spaces. Diagnosis: Miliary Tuberculosis of Liver." (Fig. 2).

The patient was started on dihydrostreptomycin in a dosage of 0.5 gm. twice daily. The temperature gradually returned to normal. His appetite improved and he gained 12 lbs. in weight. Repeated chest film revealed no change. Streptomycin was discontinued after the 12th week, and patient was discharged improved on the 163rd hospital day. He is to be followed at intervals in the future.

CASE 3. This 22 year old negro male entered the hospital on September 14, 1949, complaining of jaundice and abdominal discomfort of 5 months' duration, associated with a 10 lb. weight loss. For several days prior to admission he had noted clay colored stools and dark urine.

Physical examination revealed icteric sclerae and a tender liver, palpable 2 fingers' breadth below the right costal margin. His temperature was 101.4°F.

Laboratory studies. Complete blood count, urine, and blood Kahn test were normal. The urine was positive for bile and urobilin in dilution 1:10. Stools were positive for urobilin. Liver function tests revealed negative cephalin flocculation, icteric index of 80, total proteins 9 gm. per 100 cc., prothrombin time 27 seconds with a 15 seconds' control and alkaline phosphatase 17.9 B.U. The prothrombin time after 3 days of parenteral vitamin K

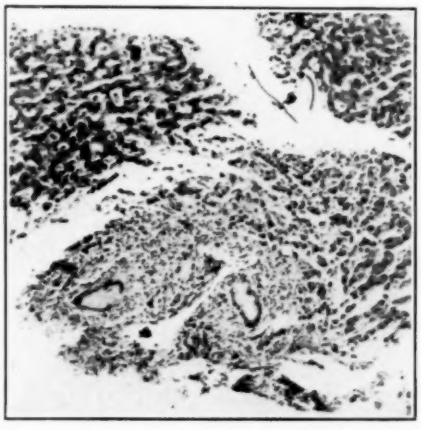

FIG. 1. CASE 1.—Photomicrograph showing a periportal granuloma containing a large Langhans' giant cell. There is a small bile duct adjacent to the granuloma (x 110).

FIG. 2. CASE 2.—Photomicrograph showing 2 adjacent granulomata, one of which shows a Langhans' giant cell near the periphery (x 110).

therapy was 15 seconds with a control of 15 seconds. Sputum examinations and gastric washings were negative for acid-fast bacilli. PPD skin test (0.00002 mg. strength) was positive. An initial chest film was negative. Intravenous pyelograms were negative. Urine cultures were negative for acid-fast bacilli. Spinal fluid studies including culture and guinea pig inoculation were negative for tuberculosis.

The clinical picture and laboratory data suggested an extrahepatic obstructive jaundice. Exploratory laparotomy was being contemplated when a needle biopsy of the liver was done. The pathological report was as follows: "Section of liver shows a few small focal tubercles composed of an outer ring of lymphocytes, then epithelioid cells which surround a core of pink, granular, acidophilic, caseous material. A giant cell is seen in one of these areas. An occasional canaliculus is plugged with inspissated bile. Diagnosis: Miliary Tuberculosis of Liver." (Fig. 3).

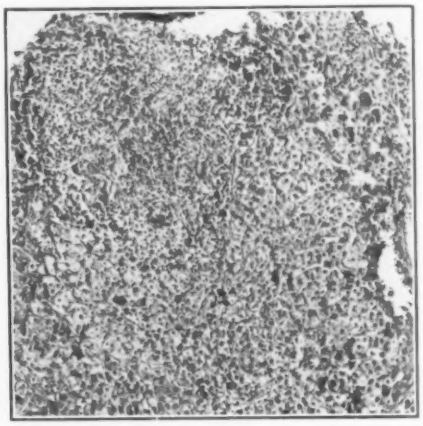

FIG. 3. CASE 3.—Photomicrograph showing an intralobular granuloma with a small Langhans giant cell at the periphery (x 110).

Subsequent peritoneoscopy revealed tubercles on the left lobe of the liver and on the spleen. The veins on the anterior peritoneum around the umbilicus were enlarged, suggesting an early portal hypertension.

After the biopsy, dihydrostreptomycin therapy was begun in dosage of 1 gm. twice daily and has been continued for 12 weeks.

There has been a 6 lb. weight gain. However, the icterus has not shown any decline and the febrile course continues. A recent chest film showed a questionable miliary spread throughout both pulmonary fields.

Discussion. Tuberculosis of the liver occurs in 3 anatomical forms: 1, miliary tuberculosis; 2, massive tuberculomata; and 3, tuberculosis of the bile ducts[1]. All 3 of the cases reported are believed to be of the miliary type. In the one autopsied case it is believed that the hepatic tuberculosis resulted from pylephlebogenous spread from intestinal tuberculous lesions.

The first patient had been ill for 2 years prior to the establishment of the diagnosis and the institution of streptomycin therapy. He was severely debilitated at the time that therapy was instituted and lived only 10 days thereafter. It is not felt that this case represented a fair therapeutic trial. One can only speculate as to the outcome of the disease in this patient, had the diagnosis been established earlier.

The second patient had been ill for 1 year when the diagnosis was established. A favorable response to dihydrostreptomycin seems definite. Therapy was continued for 3 months. At the time of discharge he had been afebrile for ten weeks, he had gained 12 lbs. in weight, and was asymptomatic. There were no toxic manifestations from dihydrostreptomycin.

The third patient has shown no definite improvement. Streptomycin in this case will be continued, but its value is open to serious question. It is of special interest that the findings prior to needle biopsy of the liver were such that surgical exploration was being contemplated. Securing a specimen of liver tissue for histologic study was most important in establishing the presence of parenchymal hepatic disease.

Other authors have made note of the importance of miliary tuberculosis of the liver and have prophesied that awareness of this diagnostic possibility would lead to more frequent recognition. Herrell and Simpson[4] suggested that one of the causes for recurrent idiopathic hyperpyrexia is tuberculous involvement of the liver. Warthin[11] emphasized that the clinical picture resembled an acute typhoid state, and one of his cases had been previously mistakenly diagnosed as Weil's disease. It is significant that these 3 cases were found in a relatively small series of 140 liver biopsies done on a general medical service. This would seem to indicate that the disease entity is not infrequent.

We believe that the employment of needle biopsy of the liver is an important addition to the diagnostic armamentarium in miliary tuberculosis of involvement of the liver. Craddock and Meredith[2] also have recently noted the value of punch liver biopsy in the diagnosis of miliary tuberculosis. It is noted by other investigators[6,9,10] that tissue obtained by aspiration needle biopsy of the liver affords precise information concerning structural changes in this organ. We realize that any pathological process of localized or patchy distribution may make a true evaluation difficult. Only a positive microscopic finding is significant. Despite this obvious drawback, it has been possible in the presence of an enlarged liver to diagnose in these 3 cases miliary tuberculosis by microscopic study of material obtained on needle biopsy of the liver. The actual technique of needle biopsy of the liver has been adequately presented by previous authors.

Conclusions. 1. Three cases of miliary tuberculosis of the liver have been presented in which the diagnosis had not been established clinically, bacteriologically, or radiographically prior to needle biopsy of the liver.

2. Needle biopsy of the liver is suggested as a relatively safe and simple procedure to aid in the diagnosis of miliary tuberculosis of the liver.

3. It is suggested that miliary tuberculosis of the liver may be a more common disease entity than is clinically recognized.

4. The response to streptomycin was gratifying in only 1 of these 3 cases of miliary tuberculosis of the liver.

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POLAROGRAPHIC STUDIES OF PROTEINS AND THEIR DEGRADATION PRODUCTS

III. THE POLAROGRAPHICALLY DETERMINED PROTEIN INDEX IN ARTHRITIC AND OTHER DISEASES*

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One of the greatest difficulties in the application of Brdička's polarographic protein reaction[1] to the study of disease has been the lack of (a) suitable standards for comparison and (b) a uniform way of expressing the results in order to facilitate a comparison of data obtained by different workers. This becomes especially apparent if the deviations from normal are not as large as in cases of advanced cancer where Brdička first noticed them[2]. Even in these the results published may be influenced to some extent by the enthusiasm of the worker. For instance, in looking over the data obtained by some 20 authors in over 2000 cancer cases[6] one finds positive reactions reported for as little as 54% and as high as 100% of the cases tested. While these differences in the effectiveness of the reaction may in part be due to modifications in technique, they undoubtedly reflect to some extent the values which were taken by the different investigators as their upper limit of normal.

As a rule, the polarogram of a "normal" serum was taken as a basis of comparison, but any single serum specimen is a poor source of reference since the "normals" vary among themselves. C. Tropp[12], who demonstrated a correlation between the polarographic curve and the protein concentration of normal serum, abandoned attempts to work out a satisfactory standardization of normals on the basis of this correlation and chose the wave height of a standard cystine solution as reference for all protein curves. However, even this cystine solution was not completely satisfactory because it showed aging effects which were very rapid once it was added to the alkaline cobalt test solution. Köhler[8] preferred the wave height of a standardized preparation of a commercially available peptide as reference for his serum analyses, but this, it seems, would be very difficult to duplicate by others.

This lack of basic standards plus the fact that the technique is not very simple and requires special equipment are sufficient reasons why the method has not yet become widely used in this country. Attempts to improve the sen-

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sitivity and specificity of the polarographic reaction for application in cancer diagnosis have so far not been very successful. From our own observations, we are led to believe that the change in blood proteins which is made apparent by this test must be a general response of the body to various kinds of stress. Trial applications of the technique carried out by Walker and Reimann[13], and by Klatt, Rusch, Dirksen and Meloche[7] have more or less confirmed the findings of European workers and made obvious the need for more basic research.

Realizing these complications, we first set out to simplify the technique. Secondly, we had to find the basic relationship between the observed current and the characteristics of the dropping mercury electrode. This was needed to make possible a comparison of results obtained with different capillaries, since the Ilkovič equation[9] is not applicable to the type of current obtained in these studies. It became further necessary to develop a set of standards which would permit the evaluation of small deviations from the normal on a statistical basis, after preliminary tests showed that the changes in the polarographic protein reactions in the arthritic diseases studied here are on the whole much smaller than in cancer. We feel we have accomplished these purposes (a) in the development of the protein index[10], (b) by expressing our currents in microamperes/mm^2. of electrode surface[11], and (c) by establishing the normal distribution curve for the protein index and for the factors which determine its value[11].

It is the purpose of this paper to illustrate the application of these improvements in technique to a study of arthritic diseases and some other abnormalities. We have been able to analyze our results statistically and thus establish even small but significant differences between normal individuals and patients with different diseases. We feel that such an approach will help to evaluate the possible applications of this technique which may eventually aid in an understanding of the important disturbances in the protein metabolism which undoubtedly occur in many diseases.

Methods. The procedure for obtaining the polarographic "protein index" has been described in detail in the second paper of this series[11] and needs only to be outlined here. The protein index is a function of the ratio of the polarographic wave heights obtained with 2 different tests, the *filtrate* and *digest* tests. These tests are carried out on 0.5 ml. samples of oxalated plasma which have been digested with potassium hydroxide for 30 minutes. For the *digest test* some of the alkaline digest is added to a cobaltous chloride solution containing ammonium hydroxide and ammonium chloride as a buffer; it is then polarographed immediately. For the *filtrate test*, proteins remaining in the alkaline digest are first precipitated with sulfosalicylic acid and then filtered off after an interval of 10 minutes. The resulting filtrate contains some polypeptides which produce a polarographic curve when added to a solution of hexaminocobaltic chloride containing ammonium hydroxide and ammonium chloride as a buffer. The wave height obtained in the latter case (the filtrate wave) is divided by that obtained in the former test, (the digest wave). This ratio, multiplied by a factor which depends on the galvanometer sensitivities used for the 2 tests then gives us the protein index[11]. If these 2 tests are carried out under identical experimental conditions on the same plasma sample, the protein index becomes practically independent of small variations in room temperature and of the characteristics of the capillary used for the dropping mercury electrode. In our opinion these 2 features make the technique suitable for routine use *in the clinic*. However, for the research laboratory we recommend: (a) work at nearly constant, known temperature, and (b) a more careful analysis of each test by evaluation of the wave heights in microamperes/mm^2. of electrode surface. The additional information thus gained may be of value, as will be shown later.

The data given in this paper have all been

evaluated in this way and thus permit a statistical analysis and comparison with the previously reported normal values[11]. These are 16.7 ± 0.17 microamperes/mm². for the digest test, 2.72 ± 0.18 microamperes/mm². for the filtrate test and 2.4 ± 0.17 for the protein index, with standard deviations of 0.97, 1.01, and 0.95 respectively. Abnormal plasmas usually produce higher wave heights in the filtrate test and lower wave heights in the digest test than normal plasmas; a combination of the 2 tests in the protein index may therefore magnify these deviations from normal and make the polarographic analysis more sensitive.

The test as it is used at present is of course still empirical because we do not know the exact nature of the substances in the blood responsible for the curves. The results obtained with such empirical tests may, nevertheless, be of considerable value, since many clinical tests are used successfully without a clear understanding of the exact mechanism. Brdicka[1] who first discovered these polarographic protein reactions has ascribed the observed currents to the reduction of hydrogen ions at the dropping mercury electrode catalyzed by the complex which is formed between cobalt and sulfydryl groups. (Any disulfidic groups are first reduced to sulfhydryl groups at the dropping mercury electrode, but the currents resulting from this reaction, which is non-catalytic, are so small in comparison with the catalytic currents as to be negligible.) Probably most of the sulphur containing groups in a protein molecule are not free enough to react polarographically; the alkaline digestion apparently causes an unfolding or rearrangement within the molecule and thus exposes a larger number of sulfhydryl groups while it splits off many polypeptides and amino acids at the same time. The digest test represents the unfolded protein molecules after alkaline digestion, while the filtrate test represents the polypeptides (probably peptones or proteoses) which have accumulated during the digestion.

Our study included the following arthritic diseases: fibrositis, osteoarthritis, gout, Marie-Strümpell disease, and rheumatoid arthritis. For purposes of comparison we have distinguished between those diagnoses in which the signs and symptoms were typical of a single disease (as far as that is possible) and those in which several diseases were listed as possibilities. As will be shown, inclusion of the latter material did not present a serious problem in the final analysis. In all of these arthritic diseases the polarographic results were so close to those found in normal individuals that only statistical analysis could reveal the existence or non-existence of deviations from the normal. An evaluation of the effect of treatment in any single one of these cases by an observation of the changes in the protein index is therefore unreliable. On the other hand such a follow-up of individual patients appears to be entirely feasible whenever the protein index values are high. This will be illustrated by a case of mumps-arthritis. In addition, data will be presented which were obtained with plasma from cancer patients before and after treatment, and with monkeys after the administration of a carcinogen. These make it seem very probable that the course of treatment of malignancies can be evaluated in individual cases by means of the protein index.

The apparatus and equipment used has been described before[11]. All experiments were carried out at room temperature which did not vary more than 5°C. In most instances duplicate analyses on each plasma sample gave results which did not differ by more than 2%.

The results obtained in the arthritic diseases have been summarized in Table 1. The tests were carried out over a period of several years and in many cases an attempt was made to follow the effect of treatment by repeated plasma analyses. The number of plasma samples listed in the Table are therefore considerably larger than the number of cases. Since the protein index of patients did not remain constant during treatment, our statistical analysis has been made on the basis of

TABLE 1.—RESULTS IN ARTHRITIC CASES

Disease		Number of Cases	Number of Plasma Samples	Digest Test: Mean Value μA. mm.² ±S.E.M.	Digest Test: Difference From Normal μA. mm.²	Digest Test: Fisher's t	Filtrate Test: Mean Value μA. mm.² ±S.E.M.	Filtrate Test: Difference From Normal μA. mm.²	Filtrate Test: Fisher's t	Protein Index: Mean Value Units ±S.E.M.	Protein Index: Difference From Normal Units	Protein Index: Fisher's t
Normal Values				16.7±0.17			2.72±0.18			2.4±0.17		
Fibrositis	(all)	13	20	15.6±0.46	−1.1	2.25	2.47±0.29	−0.35	1.03	2.4±0.31	0.0	
	(typ.)	8	11	16.2±0.29	−0.5	1.47	2.58±0.43	−0.14	0.30	2.4±0.42	0.0	—
Osteo-arthritis	(all)	16	21	15.1±0.30	−1.6	4.57	2.90±0.26	+0.18	0.56	2.9±0.24	+0.5	1.67
	(typ.)	10	13	15.4±0.44	−1.3	2.77	2.94±0.34	+0.22	0.58	2.8±0.29	+0.4	1.18
Gout	(all)	38	68	15.4±0.16	−1.3	5.65	3.64±0.21	+0.92	3.41	3.6±0.22	+1.2	4.45
	(typ.)	25	47	15.6±0.18	−1.1	4.40	3.59±0.21	+0.87	3.11	3.5±0.22	+1.1	3.93
	(acute)	7	10	15.0±0.36	−1.7	4.35	4.75±0.48	+2.03	4.23	4.7±0.46	+2.3	4.69
Marie-Strümpell Disease		14	28	15.7±0.35	−1.0	2.56	4.93±0.47	+2.21	4.42	4.8±0.50	+2.4	4.62
Rheumatoid Arthritis	(all)	75	144	14.4±0.15	−2.3	10.00	3.94±0.19	+1.22	4.69	4.2±0.21	+1.8	6.57
	(typ.)	61	111	14.5±0.17	−2.2	9.17	3.95±0.20	+1.23	4.55	4.2±0.23	+1.8	6.21
	(all f.)	53	108	14.5±0.17	−2.2	9.17	3.74±0.21	+1.02	3.78	4.0±0.24	+1.6	5.33
	(typ. f.)	42	81	14.6±0.20	−2.1	8.08	3.73±0.22	+1.01	3.61	3.9±0.25	+1.5	5.00
	(all m.)	22	36	14.2±0.30	−2.5	7.14	4.41±0.36	+1.69	4.23	4.8±0.43	+2.4	5.22
	(typ. m.)	19	30	14.2±0.36	−2.5	6.25	4.56±0.37	+1.84	4.48	5.0±0.44	+2.6	5.91
	(severe)	12	27	13.8±0.20	−2.9	10.73	4.05±0.28	+1.33	4.03	4.4±0.37	+2.0	5.00
	(mild)	11	12	14.4±0.42	−2.3	5.35	2.72±0.51	0.00	—	3.4±0.61	+1.0	1.59

typ.—typical cases

plasma samples rather than cases studied.

For comparison, the normal values have also been given in Table 1. In general all differences from normal are significant when the *ratio* of the difference to the standard error is greater than 2; whenever the differences are *highly* significant, this ratio has been italicized.

Protein Index. It becomes immediately obvious from Table 1 that the protein index in fibrositis and osteoarthritis is essentially normal and that it is definitely increased in gout, Marie-Strümpell disease, and rheumatoid arthritis. While this increase is, on the average, not very large compared to some other diseases which will be discussed later, it is highly significant nevertheless. If we compare groups (t) of cases which were considered typical of the disease with corresponding groups (a) in which cases were included which were not so typical or in which the diagnosis was doubtful, we find practically no difference in the protein index. This indicates, in our opinion, that possible errors in diagnosis cannot have had any significant effect on our results.

The difference between male and female patients with rheumatoid arthritis is statistically significant (being equal to 2.29 times the standard error of the difference). This difference was surprising since there is no difference in the protein index of normal men and women. It may perhaps mean that men who are about a third as likely to get the disease might be more severely affected once they get it. If we compare the mean values for the severe, mixed, and mild cases of rheumatoid arthritis we find protein indexes of 4.4, 4.2 and 3.4 in the expected order. However, statistical analysis shows that these differences are not significant, probably because the number of cases tested is not large enough. From this it becomes evident that a prognosis of rheumatoid arthritis by means of the protein index would be unreliable. Obviously diseases in which the protein index is so close to normal as in the above must be different in this respect from the ones in which there is a marked deviation from normal so that a return to normal during recovery can easily be observed (that is, in cancer, tuberculosis, or in the case of mumps arthritis described later).

The difference between the mean values of the protein index of typical gout cases in general and of cases during an acute attack is statistically significant. However, here too the values are so close to normal that in any individual case the results may vary unpredictably, as may be seen from the examples given in Table 2.

TABLE 2.—PROTEIN INDEX IN GOUT

	Acute Attack	*Subsided*	
Patient	*Protein Index*	*Days later*	*Protein Index*
F. O.	5.9	14	1.8
C. G.	5.2	14	2.4
P. M.	3.6	7	3.9
I. S.	3.8	148	4.0
S. W.	4.1	136	3.0

Filtrate Test. The findings in Table 1 essentially parallel those obtained with the protein index since the magnitude of the latter is largely determined by the filtrate wave. It is of interest that the mean values of the filtrate wave in fibrositis are lower than normal, whereas all other diseases studied by us show an increase above normal if there is any change at all. The amount by which these waves are lowered is too small and the number of cases not sufficiently large to make this deviation from normal statistically significant. However, out of the typical fibrositis cases, all but 2 showed this

rare negative deviation so that we feel that it may be of some significance, and might warrant further study.

Digest Test. The results shown in Table 1 also are essentially the same as those obtained with the protein index. The only difference noted is in typical cases of osteo-arthritis where the digest wave is significantly lower than normal. Although we do not have any direct evidence, we presume this to be merely a reflection of changes in total plasma protein[12].

Discussion of Results. In spite of the fact that the deviations from normal in the polarographic tests have been quite small in the above arthritic diseases, a definite difference has been found between the 2 groups, osteo-arthritis and fibrositis on the one hand and rheumatoid arthritis, Marie-Strümpell's disease and gout on the other hand. This difference is in accordance with other clinical evidence by which the 2 groups are segregated. Thus, for instance, the sedimentation rate and the albumin and globulin content of blood are normal in osteo-arthritis and fibrositis whereas the sedimentation rate is elevated in rheumatoid arthritis and Marie-Strümpell's disease, and in gout during an acute attack; furthermore the globulin content is high and the albumin content low in all 3 of these diseases[4].

We conclude therefore that the protein index is a sufficiently sensitive measure to indicate small changes in protein metabolism if a large enough series of experiments is performed to permit statistical analysis. However, the protein index at present does not appear to be suitable for following the course of a disease unless its deviation from normal is of greater magnitude than in the arthritic diseases discussed above. No systematic studies of this nature have so far appeared in the literature but the indications are that it is entirely feasible in cancer[3] and some infectious diseases[5]. To illustrate this possibility we are presenting here a striking case in which exceptionally high values of the protein index were found. This case of unusually severe arthritis following a period of swelling of the left parotid gland was diagnosed as "mumps arthritis". The details of this case will be reported elsewhere by one of us (J.S.D.); in this paper we are interested only in a comparison of the laboratory findings which are shown graphically in Fig. 1. It is clear from this Figure that there is a marked rise and fall in all 3 measured quantities; the white cell count, the sedimentation rate and the protein index. It appears that the fall in white cell count precedes that in the sedimentation rate while the latter almost coincides with that in the protein index. This raises the question whether the last 2 measurements indicate the same abnormality in the blood.

Several investigators who have compared different modifications of the polarographic protein reaction with the sedimentation rate have concluded that there is, in general, a tendency for the polarographic findings to indicate abnormality whenever the sedimentation rate is elevated. However, in nearly every case there were marked exceptions so that the sedimentation rate may be low when the polarographic reaction is high, and vice versa. In none of these studies has a statistical analysis of the correlation been attempted.

Our experiments likewise indicated that the protein index was often high when the sedimentation rate was elevated but that there were numerous exceptions, as, for instance, in pregnancies, where the protein index was practically normal[11]. We consequently performed a statistical analysis of the

correlation with 192 samples for which we had determined the protein index and the sedimentation rate (Westergren technique). We found the following correlation coefficients: 0.48 for the protein index, —0.56 for the digest, and 0.42 for the filtrate values. This would indicate that the large protein molecules, as exemplified by the digest values, are more intimately related to the sedimentation rate than the smaller polypeptides which are expressed in the filtrate values and which contribute most to the changes in the protein index.

It remains to be decided in what diseases a checkup of the treatment of a patient by means of the protein index will most likely be worthwhile. On the basis of our past experience we would place in this category any disease producing a protein index of 6 or more, that is, at least 1 unit higher than the highest normal values which

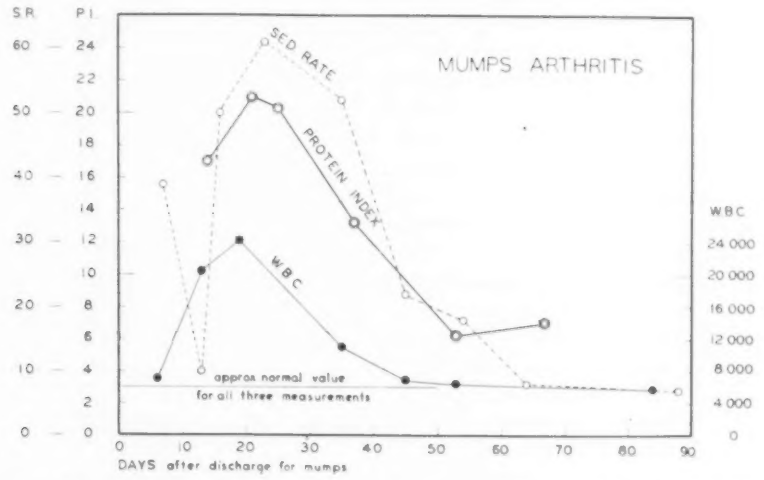

FIG. 1.—Correlation between Protein Index, Sedimentation Rate, and White Cell Count in a case of Mumps Arthritis.

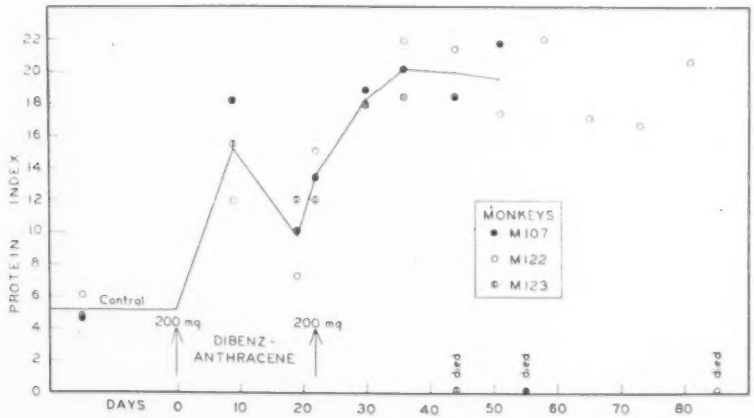

FIG. 2.—Changes in the Protein Index of 3 Monkeys after Administration of Dibenzanthracene (The drawn out curve represents the average of the results obtained).

we have observed so far. Besides cancer, this would include some of the highly infectious diseases such as pneumonia and tuberculosis. In cases of cancer even low protein index values may bear watching when they have been obtained after surgical removal of the tumor, since they may be increased by metastases. Changes in the protein index of monkeys after the administration of a carcinogen have also been large enough to justify observation of individual cases. In Fig. 2 are shown the results obtained with 3 monkeys to each of which dibenzanthracene was given in 2 doses of 200 mg.* As may be seen, their response was fairly uniform.

An idea of the values which can be expected for the protein index of cancer patients before and after treatment may be had from Table 3. It can be noted that the protein index is high in active cancer cases and shortly after removal of the tumor; it is further evident that perfectly normal as well as rather high protein index values can be found after treatment. On the basis of these figures, it seems reasonable to anticipate that it should be possible to follow the condition of cancer patients during and after treatment by repeated determinations of the protein index. Work is now in progress to evaluate this application of the protein index.

Summary. It has been shown that the protein index in gout, rheumatoid arthritis, and Marie-Strümpell disease is significantly different from that found in fibrositis and osteo-arthritis where it is essentially identical with the protein index of normal individuals. These findings are in accord with other clinical evidence by which these arthritic diseases are segregated into 2 groups. Since the observed deviations from normal in the protein index were very small, their significance could be proved only by statistical analysis of a sufficient number of samples. While this seems to establish the protein index as a sensitive indicator of small changes in protein metabolism if applied to large numbers of cases, it also demonstrates that the protein index is unreliable in diagnosis and prognosis

TABLE 3. PROTEIN INDEX IN CANCER CASES

Patient	*Sex*	*Diagnosis*	*Protein Index*
S. F.	F	Pelvic tumor	15.2
E. V.	M	Ca stomach	15.7
R. S.	M	Ca left testicle with metastases	9.9
G. E.	F	Cerebral metastases from breast adeno Ca (r. and l. mastectomy 2 and 3 years ago)	11.1
J. D.	M	Adeno Ca of sigmoid, 5 days postop.	10.0
A. H.	M	Ca caecum, 4 days postop.	8.6
S. P.	M	Ca rectum, resected 1 yr. ago	9.1
C. C.	F	Ca sigmoid, resected 2 yrs. ago	2.5
F. G.	M	Ca larynx—treated—well	2.6
R. M.	F	Benign tumor in left breast	4.9
K. B.	F	Ca breast, removed 1 yr. ago	1.5
S. M.	F	Ca breast, removed 2 yrs. ago	1.2
S. S.	F	Ca breast, removed 8 yrs. ago	6.9
M. L.	F	Malignant tumor, removed 6 yrs. ago	10.3

* Plasma samples for this study were kindly furnished by Dr. K. Dobriner of Memorial Hospital, New York.

of individual patients suffering from any of these diseases.

It is suggested that diseases in which the protein index is greater than 6 are suitable for studying the effect of treatment by observation of the protein index. Some infectious diseases and cancer, in which the protein index may be even greater than 10 belong in this category. As illustrations, marked changes in the protein index are shown in a case of mumps arthritis during recovery, and in monkeys after the administration of a carcinogen.

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THE RESPONSE TO METHACHOLINE IN RHEUMATOID ARTHRITIS*

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Increased sensitivity to methacholine, which was observed[2] in 1945 in patients with adrenal cortical insufficiency, suggested a defect in the response of the autonomic nervous system. The reported effects of the adrenocorticotropic hormone and cortisone on rheumatoid arthritis[1] prompted a study of the responsiveness to methacholine in this condition, comparing it to that seen in normal controls and in patients with other disease states.

Methods. Methacholine chloride† was administered subcutaneously in doses of 2.0 mg. to 21 ambulatory clinic patients with rheumatoid arthritis, 10 normal controls and 20 patients with varied medical disorders. The diagnosis of active rheumatoid arthritis was established in each instance by appropriate laboratory and serological tests as well as on clinical grounds. Although some of this group were receiving salicylates or gold salts at the time of the experiment, others were being given no medication.

All observations were made with the subject lying flat in bed. Blood pressures were recorded every minute for at least 10 minutes prior to the administration of methacholine and until successive readings showed no fluctuations. After injection, readings were made at ½ minute intervals until values had returned to their initial levels. The lowest systolic and diastolic values were recorded and compared to the baseline determinations. Emphasis was placed on the change in diastolic pressure because of the greater variation encountered in the systolic. The consistent similarity of the response, by means of repeated tests undertaken by different observers in subjects of each group, supported the validity of the change in diastolic pressure.

Elderly or hypertensive patients and those with myocardial disease or allergic disorders were purposely not tested because of the possibility of adverse reactions or the dangers of too great a blood pressure fall. Atropine was kept available for immediate hypodermic use.

Results. The effect on blood pressure, following the subcutaneous injection of 2.0 mg. of methacholine chloride, is shown in the accompanying table. The maximum drop in arterial tension occurred between 2 and 4 minutes after injection.

A decrease in diastolic pressure of 10 mm. of mercury or more was recorded in 20 of the 21 patients with rheumatoid arthritis. The one subject who responded to a lesser extent showed fewer clinical and laboratory signs of an active process than any other in the series. In contrast, but 2 of 10 subjects without disease and 6 of 20 patients with varied medical disorders showed a diastolic fall of 10 mm. or more. Of the 6 hyperreactors with other conditions, it is note-

† Mecholyl chloride (Methacholine Chloride U. S. P. Merck).

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worthy that 1 had lupus erythematosus disseminatus, and 3 were suffering from active viral infections.

The majority of persons tested noted varying mild sensations of facial flushing and warmth, sometimes increased salivation. Palpitations and increased peristaltic activity were remarked upon in a few instances. Visible redness of the face and neck was the only objective finding in most cases. These reactions were more severe in the group with rheumatoid arthritis.

Comment. This study indicates that patients with rheumatoid arthritis are conspicuously more sensitive to small doses of methacholine than are normal controls or patients with the other active disease processes tested. Factors such as fever, elevated sedimentation rate, weight loss, emaciation or chronic illness cannot be regarded as responsible for the difference in reaction, as these conditions were more pronounced in many of the patients with non-arthritic disorders.

The exaggerated response to methacholine in both rheumatoid arthritis and Addison's disease might be employed as an argument relating this form of arthritis to the adrenal cortex, particularly in view of the effects of hormonal therapy. It should be recalled, however, that pathological changes in peripheral nerves and autonomic dysfunction may occur in rheumatoid arthritis and that disturbances in autonomic nervous system responsiveness may be part of several disease states. Nevertheless, the ob-

TABLE 1.—EFFECT OF METHACHOLINE CHLORIDE ON BLOOD PRESSURE

Rheumatoid Arthritis			*Normals and Other Disease States.*			
Before	*After*	*Diastolic fall (mm. Hg)*	*Diagnosis*	*Before*	*After*	*Diastolic fall (mm. Hg)*
120/74	118/70	4	normal	124/80	122/78	2
126/80	106/70	10	"	104/64	100/62	2
114/68	110/58	10	"	120/78	116/74	4
118/72	112/60	12	"	100/68	90/64	4
144/86	102/74	12	"	116/82	110/78	4
128/86	118/74	12	"	120/76	110/70	6
100/70	88/56	14	"	114/76	106/70	6
140/86	124/72	14	"	110/74	104/68	6
102/64	94/50	14	"	120/64	108/54	10
134/76	112/62	14	"	106/60	98/48	12
120/68	114/52	16				
110/70	94/54	16				
118/76	110/60	16	pul. tuberc.	96/70	96/70	0
110/80	96/64	16	metast. carcinoma	100/60	96/58	2
92/62	80/46	16	gastric carcinoma	106/72	102/68	4
128/78	114/60	18	nephrosis	122/78	110/74	4
104/64	92/44	20	polio., convalesc.	120/78	104/74	4
106/66	100/44	22	exfol. dermatitis	110/60	106/56	4
100/70	70/48	22	ulcerative colitis	106/60	96/54	6
118/90	104/68	22	pul. tuberc.	110/70	104/64	6
108/68	105/40	28	subac. bact. endocard.	102/62	82/56	6
			periph. vasc. disease	142/90	124/84	6
			pyelonephritis	105/60	100/54	6
			unexplained fever	132/64	128/56	8
			regional ileitis	118/74	108/66	8
			lymphoma	104/78	84/70	8
			pul. tuberc.	118/70	110/60	10
			homol. serum jaundice	110/64	98/54	10
			tuberc. of spleen	118/66	108/54	12
			prim. atyp. pneumonia	110/70	98/48	22
			infect. hepatitis	130/64	96/42	22
			lupus erythem. dissem.	98/68	94/46	22

served results suggest that vasomotor derangements in rheumatoid arthritis should be considered further. Furthermore, the autonomic nervous system should not be ignored in the search for mechanisms of action of the adrenocorticotropic hormone and cortisone in arthritis and other conditions.

Summary. 1. The response to methacholine chloride was tested in patients with rheumatoid arthritis and compared with that observed in normal controls and in patients with other diseases.

2. A decrease in diastolic blood pressure of 10 mm. of mercury or more was recorded in 20 of 21 patients with rheumatoid arthritis, 2 of 10 normals, and 6 of 20 patients with varied medical disorders.

3. Whether the difference in responsiveness is related to a function of the adrenal cortex or the autonomic nervous system remains to be determined.

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I. ABSENCE OF INTRINSIC FACTOR FROM INTESTINAL JUICE OF PATIENTS FOLLOWING TOTAL GASTRECTOMY*

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If the "intrinsic factor" of Castle is produced solely by the stomach, then total gastrectomy should be followed regularly by the development of pernicious anemia. This was a point made by Castle himself during his early studies[2]. However, at that time such an extensive operative procedure was rarely attempted and opportunity to observe the results of gastrectomy was not available. In recent years complete removal of the stomach has often been performed. Although a few patients have developed pernicious anemia following this operation, the rarity of its occurrence has been remarkable[8,9]. Why pernicious anemia fails to occur following gastrectomy has not been satisfactorily explained. The possibility of extra-gastric sources of intrinsic factor must be considered. The work of Castle indicates that in man intrinsic factor is not produced in the gastrointestinal tract below the stomach[3]. However, Meulengracht[7] was able to demonstrate an anti-anemic effect of hog intestinal extract, and the duodenal juice of swine has been reported to produce a hematologic response in patients with pernicious anemia[5]. The purpose of our study was to determine whether upper intestinal secretion from patients with total gastrectomy would produce a reticulocyte response when administered to a patient with pernicious anemia in relapse.

Experimental Study. Three patients who had recovered from total gastrectomy for carcinoma of the stomach were used as the source of intestinal juice. None showed any evidence of pernicious anemia. With the patient fasting, a Levine tube was introduced into the jejunum and intestinal contents aspirated over a period of 3 or 4 hours. This procedure was repeated on 3 to 12 occasions for each subject. Seventy to 100 cc. of bile stained fluid were obtained at each session. The aspirated material was strained through gauze and kept in the frozen state until used. Assay of the intestinal secretion for intrinsic factor was carried out by administering it to a patient with typical pernicious anemia in relapse. On each day that the intestinal juice was administered, a portion was thawed, mixed with 200 gm. of uncooked beef, acidified with HCl to pH 3 and incubated at 37°C for 4 hours. The mixture was then neutralized with sodium hydroxide and fed to the patient with pernicious anemia. A total of 1600 cc. of intestinal juice was given over a period of 6 consecutive days. Daily reticulocyte counts and other blood studies were performed. The results of this experiment are shown in Table 1. The intestinal juice produced no hematologic response whatever during 14 days of observation. The patient was then given intramuscular injections of liver extract. This therapy was followed 3 days later by a rise in reticulocytes, with a peak of 12.3% on the

* Experiments are now under way to explore the effects of administering vitamin B 12.

TABLE 1.—HEMATOLOGIC EFFECTS OF ADMINISTRATION OF INTESTINAL JUICE AND SUBSEQUENTLY OF POTENT LIVER EXTRACT TO A PATIENT WITH PERNICIOUS ANEMIA.

Date	*Therapy*	*Reticulocytes* %	*Hematocrit* %	*Hemoglobin* gm./100 cc.	*Red Cells* (*millions*)
12–5–47	Control period	0.2	27.9	9.0	2.58
12–9–47		0.4	29.2	9.7	2.59
12–11–47		1.4	30.0	10.0	2.48
12–12–47	1600 cc.	0.4	25.2	—	—
12–13–47	intestinal	0.4	25.4	—	—
12–15–47	juice	0.5	27.0	10.0	2.42
12–16–47		0.8	23.0		
12–17–47		1.0	28.0	—	—
12–18–47		0.4	28.0	—	—
12–19–47		—	27.0	8.6	2.28
12–22–47		1.4	27.2	8.6	2.43
12–23–47		0.8	26.0	—	—
12–24–47		0.8	26.0	8.1	2.23
12–26–47		0.7	30.0	9.5	2.54
12–27–47	Liver	2.9	26.2	8.5	2.15
12–29–47	extract	12.3	32.0	10.1	3.04
12–30–47	intra-	11.2	31.8	10.0	3.00
12–31–47	muscularly	6.8	31.9	10.2	2.67
1–2–48		3.6	33.1	10.4	3.08
3–1–48		—	41.8	14.3	5.12

5th day, and subsequent complete hematologic remission.

Comment. The failure of rather large quantities of human upper intestinal secretion to produce a hematologic response in a patient with pernicious anemia was demonstrated. The lack of response indicates that intrinsic factor, if present in intestinal secretion, is in too low a concentration to be detected by this method. We are aware that the intestinal juice contained bile, which was probably a major component of the material aspirated. This experiment does not exclude the possibility of extra-gastric source of intrinsic factor, but if such a source exists in human subjects it remains to be demonstrated. Goldhamer[4] was able to produce a reticulocyte response in patients with pernicious anemia by administering large quantities of gastric juice from other pernicious anemia patients. His observations suggest that there is a low concentration of intrinsic factor even in the gastric juice of pernicious anemia. Our studies indicate that if intrinsic factor is present in intestinal secretion, its concentration must be lower than that in the gastric juice of patients with pernicious anemia.

It is possible that the factor of time is concerned with the low incidence of pernicious anemia following gastrectomy[6]. The one patient observed in our clinic who developed pernicious anemia following gastrectomy went 7 years following the operation before symptoms developed. In some of the other reported cases the latent period has also been prolonged[1]. It is well known that following withdrawal of liver therapy, some patients with pernicious anemia may go for very long periods of time without recurrence of anemia[10]. It is not improbable that normal individuals have a much larger store of EMF and can be deprived of intrinsic factor for even longer periods of time without detrimental effect. Recent demonstration of the remarkably minute quantity of liver factor (vita-

min B 12) required for maintenance of a normal blood picture in pernicious anemia[11] lends credence to the theory that existing stores of EMF may be sufficient to maintain patients for years following gastrectomy. At the present time there are few patients with total gastrectomy who have been observed for 5 years or more[1]. It will be of interest to study these patients as they become available to determine whether they will eventually develop pernicious anemia.

Summary. Intestinal juice aspirated from the jejunum of patients following total gastrectomy produced no hematologic response when administered in large quantities to a patient with pernicious anemia.

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PERIARTERITIS NODOSA SIMULATING EOSINOPHILIC LEUKEMIA* A CASE REPORT

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In eosinophilic leukemia there may be a preponderance of mature eosinophils in the peripheral blood and bone marrow with little infiltration of organs. Because of its rarity and this dissimilarity from the more common types of leukemia, the existence of eosinophilic leukemia as a nosologic entity has been questioned[6,12]. In 1944 Friedman, Wolman and Tyner[9] collected 12 cases of eosinophilic leukemia from the literature, adding a case of their own. Since then, scattered case reports of eosinophilic leukemia[1,2,7,8,10,12] have appeared. Hodgkin's disease[5,17], periarteritis nodosa[11,13,18], and trichinosis[16] as well as less clearly understood febrile illnesses[3,15] can produce a predominantly eosinophilic leukocytosis of a magnitude suggestive of leukemia, and may be characterized by a preponderance of mature eosinophils in peripheral blood and bone marrow with moderate infiltration of other organs. Thus, the findings of large numbers of eosinophils in the circulating blood may obscure rather than define a particular diagnostic problem. The following case is considered illustrative.

Case Report. Except for transient joint pains 3 years previously, M. W. (CVAH #7118), a 22 year old, white, married female, was in good health until the onset of severe menorrhagia 6 months before hospital admission. Following a sore throat 3 months later, she complained of weakness, vomiting, backache, fever, swelling of the face and eyelids, and aching, hot, tender wrists, elbows and knees. At this time at another hospital the leukocyte count was 65,000, with 75% eosinophils. Eight nucleated red cells per 100 white cells were observed. Three weeks prior to her admission to Cushing Veterans Administration Hospital, widely scattered purpuric spots appeared in the skin and shortly thereafter she complained of severe left upper quadrant pain lasting several days. Her weight fell from 125 to 105 pounds and at times she appeared to be mentally confused.

Soon after admission, the patient complained of dizziness, was incontinent of urine and became unresponsive. The temperature was 103° and the blood pressure 110/70. Pupillary reactions and optic fundi were normal. There were numerous petechiae over the trunk, extremities, face and conjunctivae. The heart was normal. The liver and spleen could not be felt. There was no lymphadenopathy. Right hemiplegia with aphasia was present.

Laboratory Findings included: red blood cells, 4 million per c.mm.; hemoglobin 12.0 gm. per 100 cc.; leukocytes 102,000 per c.mm. of which 14% were polymorphonuclear neutrophils, 9 lymphocytes, 2 monocytes, 75 eosinophils. Of the eosinophils, 58 were segmented forms, 15 band forms and 2 metamyelocytes. Platelets were slightly reduced on smear and by direct count numbered 101,000. Urinalyses were negative, the specific gravity varying from 1.011 to 1.034. Tourniquet test showed 5 petechiae per sq. cm. Coagulation time, bleeding time, clot retraction, blood

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non-protein nitrogen, serum calcium, phosphorus, alkaline phosphatase, uric acid. Wassermann, total protein, albumin, globulin, cholesterol, and creatinine were within normal limits. No ova or parasites were found on repeated stool examinations. Skin and complement fixation tests for trichinosis were negative. The heterophil agglutination was positive in 1:56 dilution. The chest, pelvis, skull, and long bones were normal by Roentgen-ray examination. Sternal bone marrow aspiration showed marked infiltration with eosinophils; the differential count of the marrow smear revealed eosinophils 15, eosinophilic band forms 16, eosinophilic metamyelocytes 19, eosinophilic myelocytes 1, neutrophilic myelocytes 2, lymphocytes 6, pronormoblasts 3, normoblasts 3.

During the first 2 hospital weeks there was low grade fever and the neurologic manifestations persisted. Thereafter she became more alert, and her hemiplegia and aphasia were slightly improved. Leukocyte count varied from 60,000 to 100,000 per c.mm., the eosinophils from 64 to 90%. Neither the injection of 25 mg. of adrenocorticotropic hormone (ACTH) nor a course of combined bacterial vaccine containing several types of organisms produced any significant variation in leukocyte count or eosinophilia. Six weeks after admission the patient suddenly became comatose and exhibited signs of a left hemiplegia. Within the next few days, the blood pressure, which had previously been normal, rose to 220/140 and scattered patches of exudate and fresh hemorrhages appeared in the ocular fundi. The blood non-protein nitrogen rose from 28 to 70 mg. per 100 cc., the leukocyte count fell from 64,000 with 66% eosinophils to 38,000 with 3% eosinophils. The patient died quietly without regaining consciousness.

Necropsy showed the characteristic lesions of periarteritis nodosa involving the arteries of the heart, kidneys, liver, spleen, lymph nodes, gall bladder, Fallopian tubes, and brain, with an area of encephalomalacia of the left cerebral hemisphere and a massive fresh hemorrhage into the right cerebral hemisphere, both due to destruction of cerebral vessels by periarteritis nodosa. The bone marrow post mortem, as in life, was filled with eosinophils in all stages of maturation.

Comment. Without necropsy the diagnosis would have been eosinophilic leukemia. In 1942, Engback, Heerup and Thomsen[6] reported a chronic fatal illness in a 7 year old boy associated with leukocytosis of 101,200 with 78% eosinophils in whom necropsy showed changes in the lung similar to those in animals dying of anaphylactic shock and changes in the arteries consistent with periarteritis nodosa. In reviewing 13 reported cases of eosinophilic leukemia, they found evidence of arterial involvement in 5, of which 3 showed severe myocardial damage. Only 2 of the 13 cases showed what they considered true leukemic changes in the organs and myeloblastic change in the blood picture. Svanberg[13] described a necropsied case of periarteritis nodosa with asthma and a leukocytosis as high as 75,000, with 75% eosinophils. He reviewed 14 reported cases of eosinophilic leukemia and believed that in one and possibly a second case, the diagnosis of periarteritis nodosa had been overlooked. Of the 6 recently reported cases of eosinophilic leukemia, 1 had asthma. There was no mention of the arteries in the pathological reports of 2 of the 3 autopsied cases.

Summary. A patient presenting clinical, peripheral blood and bone marrow findings consistent with a diagnosis of eosinophilic leukemia, at necropsy exhibited the characteristic changes of periarteritis nodosa.

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TISSUE CULTURE TECHNIQUES IN THE STUDY OF ANIMAL VIRUSES

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IT has not yet been conclusively shown that multiplication of viruses occurs in any medium except the living cell. Accordingly, the unequivocal demonstration of viral increase *in vitro* has been possible only when viable cells have been included in the system. The general recognition of this fact early led to the application of the techniques of tissue culture to the propagation of viruses. As the general methods of tissue culture, however, were developed and presented by earlier investigators, such as Carrel and his school, and by anatomists interested primarily in the genesis and morphology of cell types, they appeared extremely complex to many virologists. The latter, therefore, often hesitated to apply the techniques of tissue culture to problems involving the growth, the pathogenicity and the immune reactions of viruses. Indeed, it was not until the Maitlands (1928) showed that vaccinia virus could be grown in a simple medium consisting of fragments of kidney tissue suspended in a mixture of serum and inorganic salts that the possibility of the tissue culture technique as applied to viruses began to be widely explored. Since that time many of the viruses known to cause disease in animals have been maintained *in vitro*. In some cases their effects on the cells have been analyzed and in a few instances the influence of specific antibody on the multiplication of the virus has been investigated. Mutations apparently induced in viruses as the result of the altered environment have been observed. More recently tissue culture techniques have been applied

to the determination of the effect of various chemical compounds including antibiotic agents on the multiplication of viruses and to attempts to analyze the mode of action of certain of those that have exhibited an inhibitory effect.

In this review, the various methods of tissue culture employed in the propagation of viruses will be briefly described and the means of demonstrating the fact that growth of the agents has occurred and certain of the conditions affecting its rate will be summarized. The differences in the behavior of viruses multiplying in tissue culture will then be outlined. Finally, the more significant applications of the technique which have so far been made to the study of the properties of viruses and the control of the infections they induce will be discussed and certain suggestions for the use of these tools in future investigations will be presented. Earlier reviews[31,104,138,158,167,168], which we have made use of, give further details about the foregoing topics. In those by Hallauer[31] and Sanders[138] tables are included, summarizing the viruses which had been cultivated *in vitro* up to the time of publication. We include here a similar tabulation in Table 1 with the purpose of bringing these earlier summaries up to date. In Table 2 certain agents reported to have been grown in culture are listed. We believe that further study is required before their cultivation can be accepted without reservation.

Methods of Tissue Culture. The first attempt, apparently, to grow a virus in cells removed from the animal body was made in 1906 by Aldershoff and Broers[1]. They inoculated the cornea of a freshly killed rabbit with vaccinia virus. Then the cornea was removed, placed on gauze moistened with saline and maintained in a warm chamber for 48 hours. At the end of this time they were able to demonstrate the inclusion bodies of Guarnieri in corneal cells—an indication that viral proliferation had occurred. Whether or not this crude procedure can be regarded as the first use of tissue culture for the propagation of a virus is debatable, and accordingly the credit for this is usually given to Steinhardt, Israeli and Lambert[149] who, in 1913, applied the plasma hanging drop tissue culture technique developed by Harrison[62] in 1907, to the cultivation of the virus of vaccinia.

Harrison's procedure, which, with slight modifications, is still employed for special purposes in the study of the growth of viruses *in vitro*, consists in placing a small fragment of tissue in a drop of plasma. The plasma is coagulated by the thromboplastin derived from the fragments or by the addition of a small quantity of tissue extract. If the preparation is made on a cover glass it may then be inverted over a hollow ground slide rimmed with petrolatum to prevent evaporation. Migration or growth of cells can be readily observed upon microscopic examination. Such preparations after inoculation with a virus may be easily fixed, stained and examined for the presence of inclusion bodies or other changes in the tissue cells induced by the presence of the virus. By inoculating material from one culture to another, serial passage of a virus may be accomplished.

The most useful of the modifications of this technique have been the flask method and the roller tube method. Carrel[104] devised special round flat-bottomed flasks in which the tissue fragments are embedded in a thin layer of plasma and covered by another layer of fluid containing nutritive materials. In such flasks, which are closed with stoppers, the gaseous phase can be altered at will. Carrel's modification permits the systematic analysis of conditions and factors requisite for the growth of cells and also provides a means of maintaining the cultures for longer periods of time. He employed the method in a study of the effect of the Rous sarcoma virus on different types of cells (monocytes and fibroblasts) derived from fowls or fowl embryos[19,20,21].

Carrel[18] also devised the so-called roller tube method of tissue culture, but it was not used for the cultivation of viruses until much later (1939) when Gey and Bang[53] showed that it was possible to maintain the virus of lymphogranuloma venereum for long periods in cultures of this sort. The method consists in imbedding fragments of tissue in a thin layer of plasma spread over the inner surface of a test tube. A small quantity of nutrient fluid is added which usually consists of a balanced solution of inorganic salts, tissue extracts and serum. The tube is then placed

TABLE 1.—A SUMMARY OF THE MAMMALIAN VIRUSES THAT HAVE BEEN PROPAGATED IN TISSUE CULTURE

Virus	*Tissue*	*Type of Culture*	*Author*	*Year*
Anopheles A and B Viruses	Chick embryo	Suspended cell	Roca-Garcia[134]	1944
Columbia SK	Mouse and Guinea pig embryo brain	" "	Jungeblut & Sanders[74]	1940
		" "	Sanders & Jungeblut[142]	1942
Durand's Virus	Chick embryo	" "	Findlay[47]	1942
Ectromelia	Mouse embryo–adult mouse kidney Chick embryo	" "	Downie & McGaughey[39]	1935
Epidemic keratoconjunctivitis	Mouse embryo	" "	Sanders & Alexander[140]	1943
Equine Encephalitis	Chick embryo	" "	Olitsky, Cox & Syverton[100]	1934
" "	" "	" "	Sanders & Molloy[143]	1940
Foot & Mouth Disease	Guinea pig embryo skin & lung Adult guinea pig testis	Plasma drop	Hecke[64,65,66]	1930/31/32
" " "	Guinea pig embryo skin	Carrel	Maitland & Maitland[87]	1931
Fowl leukosis	Chicken fibroblasts	"	Doljanski & Pikovski[42]	1942
Fowl plague	Chick, pigeon, duck, goose embryo	"	Hallauer[61]	1931
" "	Chick embryo	Suspended cell	Plotz[110,113]	1932/1937
Fowl pox	Chick embryo brain & skin	Carrel	Findlay[46]	1928
" "	Pigeon spleen & bone marrow	Plasma drop	Loewenthal[81]	1928
" "	Chick embryo	Carrel	Bierbaum & Gaede[13]	1935
Herpes simplex	Rabbit testis	Plasma drop	Gildemeister, Haagen & Scheele[54]	1929
" "	"	" "	Parker & Nye[103]	1925
" "	Rabbit cornea	Tube clot	Rivers, Haagen & Muckenfuss[127]	1929
" "	Rabbit testis	Suspended cell	Andrewes[5]	1930
" "	Chick embryo	Agar slant	Cheever[24]	1939
Hog cholera	Swine bone marrow spleen, choroidplexusandlymphnode	Plasma drop Carrel	Hecke[67]	1932
" "	Swine testis	Suspended cell agar slant	Ten Broeck[151]	1941
" "	Chick embryo	Suspended cell	Boynton, Takahashi, Woods & Walker[17]	1948
Ilhéos encephalitis	" "	" "	Koprowski & Hughes[78]	1946
Infectious avian encephalomyelitis	" "	" "	Kligler & Olitsky[77]	1940
Influenza	" "	" "	Magill & Francis[83]	1936
"	Chick embryo amniotic and allantoic membrane Chick embryo brain	" "	Weller & Enders[164]	1948
Japanese B Encephalitis	Chick embryo liver and heart	Plasma drop	Haagen & Crodel[59]	1938
Kikuth's Canary Virus	Chick embryo	Suspended cell	Herzberg[68]	1933
Louping Ill	" "	" "	Rivers & Ward[129]	1933
Lymphocytic choriomeningitis	" "	" "	MacCallum & Findlay[99]	1940
Lymphocytic choriomeningitis	Mouse embryo	" "	Casals-Ariet & Webster[23]	1940
Lymphogranuloma Venereum	Mouse testis, brain and spleen Chicken embryo chorioallantoic membrane	Hollow ground slide	Miyagawa *et al.*[98]	1930
" "	Fibroblasts	Roller tubes	Gey & Bang[52]	1939
" "	Guinea pig embryo brain Chick embryo	Suspended cell	Sanders[139]	1940
Mumps	Chick embryo amniotic membrane	Suspended cell	Weller & Enders[164]	1948
Newcastle Dis. Virus	Chick embryo	Carrel	Topacio[155]	1934
" " "	" "	Suspended cell	Bankowski & Boynton[8]	1948
Poliomyelitis	Human embryonic brain and cord	" "	Sabin & Olitsky[136]	1936
"	Human embryonic brain, intestine & skin and muscle	" "	Enders, Weller & Robbins[42]	1949
"	Human foreskin	" "	Weller, Robbins & Enders[166]	1949

TABLE 1.—A SUMMARY OF THE MAMMALIAN VIRUSES THAT HAVE BEEN PROPAGATED IN TISSUE CULTURE.—(*continued*)

Virus	*Tissue*	*Type of Culture*	*Author*	*Year*
Pseudorabies	Rabbit testis	Suspended cell	Traub[156]	1933
	Guinea pig testis	" "		
	Chick embryo			
Psittacosis	Mouse spleen	Plasma drop	Bedson & Bland[11]	1932
"	Chick embryo muscle & skin & lung	Liquid drop	Bland & Canti[14]	1935
"	Chick embryo	Suspended cell Agar slant	Yanamura & Meyer[171]	1941
"	" "	Suspended cell	Faulkner & Andrewes[44]	1935
Rabbit myxoma virus	Rabbit monocytes	" "	Benjamin & Rivers[12]	1931
" " "	Rabbit testes, kidney, lung, lymphoid tissue, and leucocytes	Hanging drop	Haagen[57]	1931
Rabies	Rabbit embryo brain	Suspended cell	Kanazawa[75,76]	1936/37
"	Mouse embryo brain	" "	Webster & Clow[162]	1937
	Chick embryo brain			
"	Chick embryo	Floating clot	Plotz & Reagan[119]	1942
"	Mouse embryo brain	Suspended cell	Parker & Hollender[105]	1945
	Brains of mice up to 14 days of age			
Rift Valley Fever	Chick embryo	" "	Mackenzie[91]	1933
			Saddington[137]	1934
Rous sarcoma	Chicken monocytes	Carrel	Carrel[19,20]	1925/1926
	Chick embryo			
	Chicken spleen			
" "	Chick embryo fibroblasts	"	Ludford[89]	1937
St. Louis Encephalitis	Mouse embryo	Suspended cell	Syverton & Berry[150]	1935
" " "	Mouse embryo	" "	Harrison & Moore[62]	1937
	Chick embryo			
" " "	Mouse embryo	Agar slant	Pang[101]	1940
Theiler's Mouse Encephalomyelitis Strain (GDVII)	Mouse embryo brain	Suspended cell	Parker & Hollender[105]	1945
	Young mouse brain			
Vaccinia	Rabbit cornea	Humid chamber	Aldershoff & Broers[1]	1906
"	Rabbit cornea and Guinea pig cornea	Plasma drop	Steinhardt, Israeli and Lambert[149]	1913
"	Rabbit testis	Plasma drop	Parker & Nye[102]	1925
"	Rabbit cornea	Tube clot	Rivers, Haagen & Muckenfuss[127]	1929
"	Chicken kidney	Suspended cell	Maitland & Maitland[96]	1928
"	Kupffer cells	Liquid culture on lens paper	Beard & Rous[10]	1938
"	Chick embryo	Agar slant	Kurotchkin[82]	1939
"	" "	Roller tube	Feller, Enders & Weller[45]	1940
Venezuelan equine encephalitis	" "	Suspended cell	Lennette & Koprowski[83]	1945
			Koprowski & Lennette[80]	1946
Vesicular stomatitis of horses	Guinea pig embryo	Carrel	Carrel, Olitsky & Long[27]	1928
	Guinea pig bone marrow			
" " "	Chick embryo	Suspended cell	Cox, Syverton & Olitsky[37]	1933
			Olitsky, Cox & Syverton[100]	1934
Virus III	Rabbit testis	Carrel Suspended cell	Andrewes[2]	1929
" "	Rabbit testis, spleen, lymphoid tissue, bone marrow, lung, liver, ovary, kidney, and iris	Liquid drop	Ivanovics & Hyde[73]	1936
West Nile Virus	Chick embryo	Suspended cell	Lennette & Koprowski[83]	1945
			Koprowski & Lennette[79]	1946
Yellow Fever	Chick embryo	" "	Haagen and Theiler[60]	1932
" "	Mouse & chick embryo	" "	Lloyd, Theiler & Ricci[88]	1936
	Mouse & guinea pig testicle			
" "	Placenta, mouse tumor tissue	" "	Findlay & MacCallum[48]	1940

TABLE 2.—OTHER VIRUSES THAT HAVE BEEN REPORTED AS HAVING BEEN PROPAGATED IN TISSUE CULTURE*

Virus	*Tissue*	*Type of Culture*	*Authors*	*Year*
Common cold	Chick embryo	Vaseline sealed tubes	Dochez, Mills & Kneeland[28,29,30]	1931/36
			Powell & Clowes[122]	1934
Measles	" "	Floating clot	Plotz[115]	1938
			Rake, Shaffer & Jones[124]	1941
Russian spring-summer encephalitis	" "	Suspended cell	Chumakov[148]	1939
Salivary gland virus of guinea pigs	Guinea pig testis, salivary gland, ovary, and brain.	" "	Andrewes[4]	1930
Sand fly fever	Chick embryo	" "	Shortt, Pandit & Rao[140]	1938
			Shortt, Pandit and Anderson[145]	1940

*The evidence for the propagation of these agents in tissue cultures is thought by the authors of this article to require further confirmation.

horizontally in a rotating device. In this manner the tissue is repeatedly bathed in the nutrient fluid but is not continuously submerged. Because of these conditions cellular respiration appears to proceed more effectively and the tissues may be kept alive and growing for months or even years. The method has not only produced excellent growth of certain viruses such as those of lymphogranuloma venereum[53], vaccinia[45] and psittacosis[99] but has allowed the collection of data on the effect of prolonged contact between these viruses and the tissue cells.

Recently attempts have been made to substitute more physiologically inert and more stable substances for plasma as a supporting medium. Evans and Earle[43] in this country have obtained excellent growth of certain types of cells, such as fibroblasts and rat sarcoma cells, when they employed perforated cellophane membranes instead of plasma in Carrel flask cultures. Using what is essentially a modified plasma hanging drop method Wirth and Barski[170], and Barski and Maurin[9] in France have found that cells of several types can be readily cultivated on a thin film of the plastic "Formvar". Agar was substituted by Zinsser and his coworkers[172] as a support for the cells in a medium in which fragments of tissue were spread upon the surface of a solidified mixture of agar and Tyrode's solution. It has been shown that a number of viruses as well as the rickettsiae can be grown in a medium of this composition. An attempt has been made to use purified mammalian fibrinogen and thrombin to produce a coagulum for tissue cultures[120]. Whether such materials will prove as satisfactory as avian plasma, heretofore almost universally employed, is doubtful.

The application of tissue culture to the propagation of viruses was, as we have said, greatly extended by the development of the suspended cell type of culture described by Maitland and Maitland[96], and Maitland, Laing and Lyth[95]. Minced tissue is added to a shallow layer of liquid medium consisting of a balanced salt solution, such as that of Tyrode, and serum. The simplicity of this procedure, its ready adaptability to large scale cultivation and the ease with which it may be modified has led to its widespread use. For example, Li and Rivers[86] employed the technique for the cultivation of vaccinia virus but used a special type of flask and omitted the serum. Simms and Sanders[146] substituted an ultrafiltrate of serum for the crude material. The former contains no substances of large molecular size, although it retains the property of preserving cell viability characteristic of whole serum. The substitution of ultrafiltrate was found to be advantageous, since it reduced fat deposition in the cells, yielded a medium very low in protein and eliminated the possibility of including antiviral substances which might be present in the crude serum.

In making serial transfers of virus propagated in the Maitland type of culture previous workers have used as subinocula either a small portion of the overlying fluid or some of the fragments removed at the end of 3 or 4 days and macerated in the fluid. Weller and Enders[164] recently modified this procedure by withdrawing and replacing every 4 days the entire fluid phase. In this manner they have been able to maintain the tissue in a viable state for 30 to 40 days and to increase considerably the yield of virus obtained from a single culture. Subcultures were prepared after intervals ranging from 12 to 30 days by inoculating a small quantity of the cell-free supernatant fluids removed at these times. This maneuver probably enables the concentration of a slowly growing virus to increase sufficiently and so permits successful

transfer from one culture to another. Although it has not yet been conclusively demonstrated, it seems likely that cultivation of certain viruses such as poliomyelitis has been frequently unsuccessful in the past because the degree of dilution effected by rapid serial passage tended to outstrip the rate of multiplication.

While in the strict sense the following cannot be regarded as tissue culture, we wish to mention here a procedure described by Goodpasture and Anderson[55,56] for the cultivation of viruses that are pathogenic for man alone. These authors were able to graft bits of human fetal membranes and of human skin on to the chorioallantoic membrane of the chick embryo. The grafts became vascularized and many of the cells remained alive. In such preparations they obtained evidence for the growth of various viruses including that of herpes zoster. Blank, Coriell and Scott[16] have lately confirmed these observations.

In concluding these remarks on methods, it is worthy of the greatest emphasis that the inclusion of antibiotics and sulfonamides in tissue cultures has practically eliminated bacterial contamination—heretofore a major impediment to the extensive application of the method in the virus laboratory. A few reports have appeared on the use of these agents for this purpose[135,141,166], but their value perhaps has not yet been sufficiently recognized. In our laboratory the addition, as routine, of 50 units each of penicillin and streptomycin per ml. to the fluid phase of the Maitland type of culture has with very rare exceptions excluded contamination by bacteria. Moreover, it has been possible using 100 units each of these antibiotics per ml. to introduce throat washings and stool suspensions directly into this type of tissue culture. In this manner by inoculation of cultures consisting of human embryonic skin and muscle 2 strains of poliomyelitis virus have been recently isolated from feces[133]. It is, therefore, evident that not only the introduction of the use of antibiotics has rendered tissue culture a more simple and reliable procedure, but it has also broadened considerably the scope of the technique as applied to the cultivation of viruses.

Criteria for the Multiplication of Virus. The fact that the concentration of a virus may be high in the infected animal tissues used as the original inoculum of a series of cultures renders it imperative to apply certain rigid criteria to the results before the conclusion can be drawn that multiplication of the agent has occurred. The failure to evaluate their data by such standards has in the past led certain workers to assert that they had succeeded in cultivating various agents *in vitro* whereas they had simply shown that under these conditions the original virus containing material could be considerably diluted and still retain its capacity to infect experimental animals.

One or more of the following criteria may be used to determine whether multiplication of a virus has occurred: 1. The material from the last of a series of tissue cultures must contain the virus and must represent a dilution of the original virus inoculum sufficient to exceed by a wide margin the titer (expressed as a dilution) of the original virus inoculum. 2. It must be demonstrated that a significant increase in the quantity of virus inoculated has occurred within a single culture. 3. It must be demonstrated that an effect upon the tissue cells has occurred which is the result of virus proliferation.

Of these criteria, only the first has been employed by most investigators. Critical discussions of its application may be found in the monographs of Hallauer[31] and of Sanders[138]. Here it is enough to emphasize the fact that the number of successive subcultures should be adequate to render it highly improbable that any virus derived from the original inoculum has been carried along into the last of these subcultures. Assurance that carry-over of virus has not occurred is difficult to obtain; especially if fragmented tissues are included in the inocula. Certain controls which will be briefly mentioned below should always be included. These will aid in providing an index of how many subcultures may be required to show that multiplication has ensued.

It is evident that the criterion based on the dilution of the original inoculum requires considerable time and labor to fulfill. If the second criterion mentioned above can be invoked simultaneously, satisfactory evidence of multiplication may be obtained in a much shorter time. Thus, if a significant increase in the quantity of virus inoculated into each culture in the series can be revealed, the results of a limited number of cultures will leave no doubt that multiplication has occurred. An example of the application of both criteria is to be found in experiments of Weller and Enders[164] on the propagation of mumps virus, in suspended cell cultures in which the fluid phase was removed and replaced at intervals of 4 days. Their results are graphically summarized in Fig. 1. It is apparent that even in the original cultures of the series a definite increase in the virus was observed as measured both by its infectivity for chick embryos and its capacity to agglutinate red blood cells.

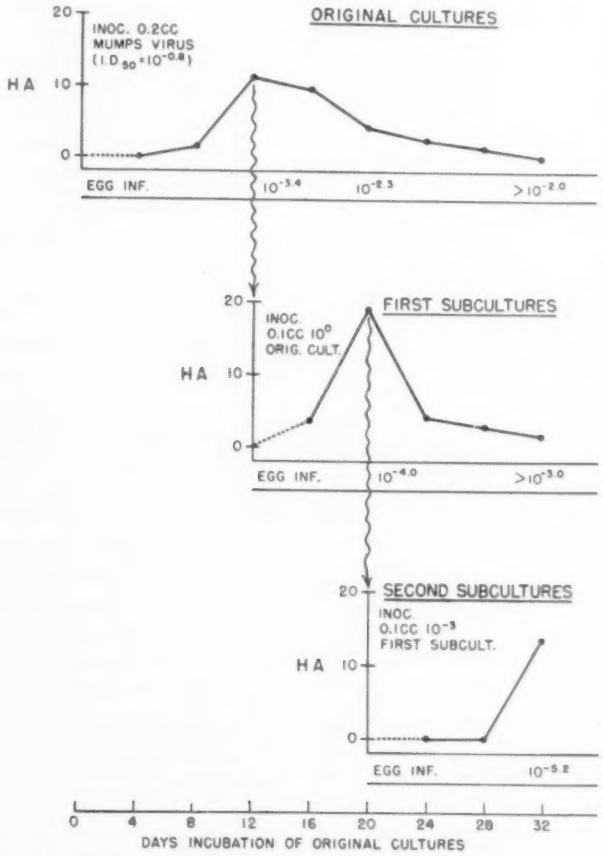

FIG. 1.—Mean hemagglutinin and infectivity titers of tissue cultures inoculated with mumps virus. The hemagglutinin titer (HA) is expressed as the reciprocal of the geometric mean of the individual titers of the supernatant fluids from 4 cultures. The infectivity titers are expressed as a dilution of the pooled supernatant fluids from 4 flasks representing the ID_{50} dose for embryonated hen's eggs calculated according to the method of Reed and Muench. (Reprinted from Proc. Soc. Exp. Biol. & Med., 69, 124, 1948, Weller, T. H. and Enders, J. F.)

The data obtained in the first and second subcultures so decisively confirmed this observation that no doubt remained as to the ability of the virus to multiply actively in the system employed.

Although both criteria, if possible, should be used in evaluating the results when a virus is cultivated for the first time or when new procedures are under investigation, it may suffice to employ only the second. Thus Gey and Bang[53] concluded that the virus of lymphogranuloma venereum multiplied in roller tube cultures of human fibroblasts, and Feller, Enders and Weller[45] found that vaccinia virus in roller tube cultures of chick embryonic tissues continued to increase during a period of several weeks. Morgan and Wiseman[99] more recently have described the active growth of psittacosis virus in such cultures without resort to the subculture of the agent. Employing suspended-cell cultures of human embryonic skin and muscle Weller, Robbins and Enders[166] have lately found that the Lansing strain of poliomyelitis virus in a culture may increase at least 1800 times within a period of 20 days.

The third criterion—the demonstration of an effect upon the tissue cells known to be the result of virus proliferation such as the formation of inclusion bodies or the appearance of widespread cellular degeneration—can only be invoked in the case of a limited number of agents. Since the effect of viruses on cells also affords a means of determining their presence in cultures this criterion will be discussed further in the next section.

As we have stated, it is essential to include several types of controls in experiments designed to reveal the multiplication of a virus. The first and most obvious is the control for contamination by bacteria or fungi which might simulate in experimental animals the effect of the agent that one is attempting to grow. Materials from tissue culture should, therefore, be inoculated as routine into suitable media. Secondly, controls are maintained to exclude the possibility that an extraneous virus has been cultivated that may have been derived either from the environment of the laboratory or from the animal furnishing the tissue. These consist of the maintenance under the same conditions of cultures which have not been inoculated with the virus that is being studied. Thirdly, controls are included to determine the period during which the inoculated virus may be detected in systems that contain: a, no tissue; b, tissues killed in some gentle manner such as by heating at 60°, Roentgen-ray irradiation or repeated freezing and thawing; c, living tissues maintained at 4° at which temperature viruses do not multiply. These controls are of the greatest importance since if the virus rapidly disappears from them while it continues to persist in the cultures of living tissue maintained at higher temperatures, it can be reasonably concluded that growth in the latter has taken place.

Methods of Demonstrating Virus in Tissue Cultures. Two general methods are available for demonstrating the presence of virus in tissue cultures. The first consists in showing that material removed from the culture exhibits an activity characteristic of the virus in question. The second is the direct or indirect revelation of some abnormal change in the tissue cells induced by the virus.

Until very recently the first method depended exclusively upon the inoculation of an appropriate experimental animal. In the case, however, of viruses like mumps and influenza which cause the agglutination of red blood cells it

has been found possible to reveal their presence in the suspended cell type of tissue culture by showing that the fluid contains hemagglutinin[164]. Within certain limits this procedure provides an extremely convenient and rapid method for detecting and measuring the virus. Measurement by the method of hemagglutination is far less sensitive than the method of testing for its infectivity. Its use, therefore, is not only restricted to those relatively few agents which induce hemagglutination, but to those situations in which large quantities of the hemagglutinating virus are present. Thus a negative test for hemagglutinin cannot be regarded as evidence for the absence of the virus. Since the viral hemagglutinin is an antigen which induces the formation of an antibody that specifically inhibits its action on erythrocytes, it is possible to identify the virus producing the hemagglutinin in tissue culture fluids by means of the hemagglutinin inhibition test.

Other viral antigens in tissue cultures may be present in concentrations sufficient to permit their detection by serologic procedures. Thus Maitland and Laing[94] in 1930 showed that an antigen was present in cultures of vaccinia virus which fixed complement with specific antiserum. Kunz[81] in our laboratory has recently determined that the mumps virus produces recognizable quantities of complement fixing antigen when grown in suspended cell cultures of chick amniotic membrane.

The most characteristic of the changes upon which the second general method depends are the intra-cytoplasmic and intra-nuclear bodies which have been observed to form in tissue cultures infected with certain viruses just as they do in the cell of the living animal. When they can be regularly produced in cultures and are typical in morphological appearance and staining properties they may be considered to result from the multiplication of the virus. Unfortunately there is often great difficulty in consistently obtaining inclusion-body formation and characteristic morphologic appearance. As indications of the presence of virus they are, therefore, not entirely reliable and have been so employed only in special instances such as in the studies of Andrewes[3,4,5] and Rivers[127,128] on the mechanism of action of antiviral sera, or those of Bedson and Bland[11] on the developmental cycle of the psittacosis virus. Furthermore the technique involved in their demonstration is somewhat complex and time consuming. When, however, no other means of demonstrating the presence of the virus are available the formation of typical inclusion bodies may be of great assistance. Thus in their attempts to grow the virus of varicella and herpes zoster in human skin grafts on the chorioallantois, Goodpasture and Anderson[56] interpreted the appearance of inclusions as evidence for the growth of the zoster virus. Formation of intranuclear inclusions following inoculation of material containing the salivary gland virus of guinea pigs into tissue cultures was observed by Andrewes[4]. Although he could not maintain the virus in serial cultures, he regarded the formation of these bodies as evidence of growth in the original series. Similarly, Weller and Kingsland[165] have recently observed the formation of intranuclear inclusions typical of those found in the lesions of varicella after inoculation of fluid from chicken pox vesicles into cultures of mixed human embryonic tissues. As yet the appearance of these bodies has not followed subinoculation of materials from the original cultures. They suggest strongly, however, that the virus of varicella has at least infected the cells in the first generation of cultures and so offer encouragement to con-

tinued effort to cultivate this strictly human pathogen *in vitro*.

As we have previously pointed out, certain viruses *in vitro* may cause marked degenerative changes in the tissue cells. The clearest example of this phenomenon is perhaps to be found in the observations made by Huang[72] in 1943 who showed that the virus of equine encephalomyelitis completely destroyed chick embryonic tissue cells within 48 hours. The lack of cell viability was revealed by the failure of these fragments exposed to the action of the virus in suspended cell cultures and later explanted in plasma hanging drops to proliferate in the latter type of medium. In 1942 Enders and Florman[40] had obtained some evidence that most of the fragments of chick tissues which had supported the growth of vaccinia virus for 3 weeks in suspended cell cultures, appeared to become incapable of proliferation when explanted to plasma hanging drops. At this time the majority of the fragments from uninoculated control cultures exhibited fibroblastic growth. Plotz and Ephrussi[117] had also observed that the virus of fowl plague led to early cell death as demonstrated by the explant method. With Weller we are at present engaged in an investigation which has already shown that both non-murine and murine adapted strains of poliomyelitis virus multiplying in suspended cell cultures of certain types of human embryonic tissues injure the cells to such a degree that cell growth in plasma explants of the fragments is completely or almost completely inhibited. Evidence of such injury is only perceptible after the lapse of about 8 days following inoculation of the virus. Histologic examination of tissue fragments which have supported the growth of poliomyelitis virus has revealed degeneration of most of the cells, provided that exposure to the virus has been sufficiently prolonged.

This method, then, of testing for cell growth by explantation appears to offer reliable and convenient information concerning the presence in cultures of certain viruses. It cannot be generally employed, however, for this purpose since it is clear that some agents cause little or no recognizable injury of the cells even after prolonged cultivation. Thus Relova and Enders[125] were unable to observe over a period of 2 weeks any definite inhibitory effect of the virus of influenza or herpes simplex on the growth of chick embryonic cells in roller tubes. Watson[160] has recently failed to find that cell proliferation of explants from suspended cell cultures of chick embryonic tissues and mumps virus was appreciably affected. Her observations are in agreement with our own based on histologic evidence concerning the incapacity of this virus to injure *in vitro* or *in vivo* the cells of chick amniotic membranes.

The extensive injury of cells in cultures produced by certain viruses is also manifested by decreased cellular metabolism. When this occurs it is readily discerned by decreased acid production. When an indicator is incorporated into the nutritive medium, a routine practice, this effect of the virus on the metabolism of the cells becomes immediately evident by comparing the pH of the inoculated cultures with that of the controls to which no virus is added. Huang[71] noted that chick embryonic tissues from 48 hour cultures of equine encephalomyelitis virus were no longer capable of reducing the pH in Ringer's solution under standard conditions. The behavior of these inoculated tissues contrasted sharply with uninoculated embryonic fragments. Previously Plotz[112] had shown that chick embryonic tissues inoculated with the virus of fowl plague and incubated for 3 days failed to

reduce methylene blue anerobically in the light. In our own studies with the virus of poliomyelitis we have found a reasonably good correlation between the presence of virus in the culture and the impaired capacity of the tissue to reduce the pH. As with the other criteria of cell injury manifested by the virus of poliomyelitis, this difference in cellular metabolism does not become apparent until the 12th to 20th day of cultivation. Nevertheless, in the propagation of these agents we have so far found this simple presumptive index of their presence extremely valuable.

Factors Influencing the Growth of Viruses in Tissue Culture. The factors that influence the growth of viruses in tissue cultures are numerous and complex. In many cases they are only partially understood; in others we have as yet no clue to their nature. The analysis of the mode of action of the known factors is, of course, rendered enormously difficult by the fact that it is often impossible to decide whether alteration of an experimental condition affects the multiplication of an agent directly, or indirectly by influencing the metabolism and growth of the tissue cells. Here we will consider briefly certain of the conditions which have been most thoroughly studied that are either essential for the growth of virus or that definitely influence it.

Biological Activity of the Tissue. With few exceptions investigators agree that living cells are an essential prerequisite for viral multiplication. From time to time there have been claims that a virus has been cultivated in media which contained no viable tissues[35,36,159]. Attempts to confirm these results have always failed. The most recent of such claims is that of Veeraraghavan[159], who reported that he was able to maintain the virus of rabies in cultures composed of heated brain. No confirmation of his work has as yet appeared.

In respect to the state of the cells there is less unanimity. It is still impossible to determine on the basis of the available data whether or not cellular proliferation is an essential condition for the multiplication of virus. Thus, although it is apparent that cell division may be reduced to a degree where it is not clearly discernible in cultures of the Maitland type, the possibility cannot be eliminated that some increase in certain cell types may occur. However, since viruses proliferate to a considerable degree under such conditions it may be concluded at least that *active* cell division is not an essential factor. Plotz and Ephrussi[118], in a study of this problem, inoculated fowl plague virus into chick embryonic tissue suspended in Drew's solution on the 5th day when mitosis was not evident, and obtained growth of this agent. On the other hand it is probable that cellular proliferation is an adjuvant in increasing the amount of virus produced. Plotz[114] found that the titer of fowl plague virus in plasma cultures of chick embryonic tissue was 100 times greater than in suspended cell cultures. In the latter he could observe no evidence of cell division as indicated by the presence of mitotic figures. Likewise Fox[50] noted that the addition of chick embryonic extract to cultures of yellow fever virus stimulated the growth of the tissue and increased the yield of virus. More information on this point is greatly to be desired. Not only is it important from the biologic point of view to be certain that increase in the amount of virus produced is a function of cell division but from the practical standpoint of obtaining greater yields of virus it is of the utmost significance.

It is the impression of most workers in the field that the multiplication of virus is dependent upon an active cel-

lular metabolism whether or not cellular proliferation is going on. But again the evidence is limited and not entirely conclusive. At first sight the experiments of Zinsser and Schoenbach[173] would seem to leave little doubt that maximal viral increase occurred at a time when the oxygen consumption of the tissues was also at its height. In tissue cultures inoculated respectively with equine encephalomyelitis virus and typhus rickettsiae and maintained in the Warburg apparatus they found that the greatest CO_2 production occurred during the active phase of viral increase. In contrast multiplication of the rickettsiae was associated with the declining phase of CO_2 output. On the basis of these observations the authors concluded that whereas viruses required actively metabolizing cells for their growth the environment of the impaired cell provided conditions suitable for the development of rickettsiae. Although evidence from subsequent investigations has supported this hypothesis in the case of the rickettsiae, it can still be debated as to whether it can be generally applied to the viruses. The virus which they studied belongs to the class which characteristically multiplies rapidly both in the animal body and in cultures. It also is one of the agents which produces rapid destruction of tissue cells. Had a more slowly growing and a less cytopathogenic agent such as the mumps virus been studied by this method, the peaks of cell metabolism and active viral increase might not have coincided so closely. In view of such considerations and the fundamental implications of the problem, it is to be hoped that further extensive investigations of this sort will be undertaken in which viruses exhibiting different growth characteristics will be included.

Amount of Tissue. In suspended-cell cultures various investigators have found that the ratio of the quantity of tissue relative to the volume of fluid must be maintained fairly constant in order to obtain maximal yields of the virus[50,130,171]. Simms and Sanders[146] for example, recommend that at least 100 volumes of fluid be added for each volume of tissue. Others[111] have found that the ratio of fluid to tissue may be varied considerably with little effect on the yield of virus provided measures are taken to prevent the medium from becoming acid. Traub[156] has described an interesting relationship between the quantity of virus inoculated and the amount of time required to initiate growth. Small inocula appeared to multiply only when relatively large amounts of tissue were present.

Type of Tissue. The multiplication of certain viruses seems to be restricted not only to tissues derived from one or a limited number of animal species but also to particular cell types. Other agents appear to be much less circumscribed in their tissue requirements. Lloyd, Theiler and Ricci[88] failed to initiate the growth of a pantropic strain of yellow fever virus in chick embryonic tissues. They succeeded when mouse tissues were substituted. According to Maitland and Maitland, the virus of foot and mouth disease multiplies in guinea pig epithelial cells but not in chick embryonic tissues. The Lansing strain of poliomyelitis virus can be cultivated in various human embryonic tissues but in spite of numerous attempts in our laboratory no evidence of growth in cultures either of chick embryonic or mouse embryonic brain has yet been obtained[85]. Additional examples of the restriction of multiplication to cells derived from susceptible hosts are given in a recent review of cellular resistance to virus infections[39].

There is little reliable information

concerning the capacity of viruses to grow in specific cell types, since the isolation and maintenance of pure lines of cells is difficult and beyond the technical range of most investigators in the virus field. One should, however, mention the experiments of Carrel[19,20,21], who propagated the virus of Rous sarcoma in chicken monocytes and believed that this agent did not multiply in chicken fibroblasts. Ludford[89] on the other hand was unable to confirm these observations and indeed arrived at the opposite conclusion. Benjamin and Rivers[12] observed that myxoma virus of rabbits multiplied in monocytes of that animal and noted that testicular tissue unless it contained these cells as a result of a previously induced inflammatory process would not support the growth of the virus. The virus of vaccinia has apparently a much wider range of cell types in which it finds the necessary conditions for multiplication. Beard and Rous[10] showed that it grew actively in pure cultures of Kupffer cells derived from the rabbit. Feller, Enders and Weller[45] obtained excellent growth in cultures consisting predominantly of chick embryonic fibroblasts, and Florman and Enders[49] observed continued production of the virus in cultures of monocytes originally obtained from rabbit's blood. Yanamura and Meyer[171] observed that psittacosis virus exhibited a similar broad affinity for cell types.

In general, as we have indicated above, the type of tissue in which a virus may be propagated corresponds with its host range, although there are rather numerous exceptions to this rule. Accordingly, when an attempt is first made to cultivate a virus *in vitro*, it is logical to select tissues from a host known to be susceptible. In this connection, however, it is pertinent to note that a number of viruses that are not pathogenic for the chicken are easily grown in chick embryonic tissues. This has led to the thought that this tissue may possess special properties which render it susceptible to infection. Although, in certain instances this may prove to be the case, in others it would seem that the high body temperature of the chicken (105° to 106°F.) is beyond that which may be tolerated by the virus. This factor probably accounts for the failure of the Melbourne strain of influenza A virus to infect newly hatched chicks, since Enders and Pearson[41] showed that it could be easily propagated in cultures of the lung tissue removed from the young birds and maintained at 37°C.

Composition of the Medium. The composition of the medium employed will depend upon the type of tissue selected and upon the virus to be cultivated. Certain agents can be maintained indefinitely in tissues suspended in Tyrode's solution alone. Serum has been added by many investigators, some of whom, like Haagen[58], thought it was essential in the cultivation of yellow fever. That it may aid in increasing the yield of virus is probable, but its indispensability is doubtful. Fox[50], for example, has been unable to confirm Haagen's observation and we are unaware of any conclusive evidence that the addition of serum is a necessary prerequisite for viral increase. Its usefulness, like that of the serum ultrafiltrate which has already been mentioned, consists in maintaining the viability of the tissue cells for longer periods of time than is possible when only a balanced salt solution containing glucose is used and in helping to preserve extra-cellular virus. Studies lately carried out by Pearson[109] and by Thompson[153] on the effect of various growth factors and vitamins have failed to reveal any substance which appears definitely to increase the yield of Theiler's mouse encephalomyeliti-

and vaccinia virus. Weller and Buckingham[163] in our laboratory have also investigated the effect of substituting the medium devised by Geiman and his co-workers[52] for the *in vitro* cultivation of malaria parasites for the salt mixture-ultrafiltrate medium we have used in our studies of the Lansing strain of poliomyelitis virus[42,166]. The former, although it contains most of the known vitamins and many of the building blocks of proteins and nucleoproteins, did not lead to a greater production of this virus.

Oxygen Supply. To insure respiration and viability of the tissue a certain amount of oxygen must be available in the system. No virus has been shown to increase in an anerobic environment with the possible exception of that of the common cold[30]. Some investigators have attempted to maintain a more or less constant tension of O_2 and CO_2. There is, however, no convincing evidence that this is essential, provided that CO_2 is not permitted to accumulate to a point where the pH falls much below 7[106]. Thus, although certain investigators[95] have considered that the surface of the fluid phase should be large and its depth shallow to afford free interchange of O_2 and CO_2 with the gaseous phase, others have been able to cultivate such typical viruses as pseudorabies[156], and vaccinia[154] and psittacosis[171], in systems in which the fluid overlay the tissue fragments to a depth of several centimeters.

Reaction of the Culture. The pH of the cultures is decreased as a result of tissue metabolism. The acidity is mostly caused by the formation of CO_2 from the splitting of the glucose in the medium. Since the tissue cells are injured by accumulation of acid, it is desirable to prevent the reaction from falling much below pH 7. In certain cases it has been shown that virus activity is impaired by relatively slight increases in hydrogen and hydroxyl ions. Sanders and Jungeblut[142], to cite a simple example, found that the greatest yields of Columbia SK virus were obtained when the pH was held at 7.5. At higher or lower values the virus was partially inactivated. Other viruses, though, seem to be unaffected over a fairly wide range of pH. In the cultivation of those which likewise severely injured the cells, change in pH has proved to be a useful index to the multiplication of the agent as we have previously indicated.

Temperature and Incubation. Cultures of animal viruses have usually been maintained at 37°C. Multiplication, however, has been found to occur at much lower temperatures. Sanders and his co-workers obtained higher concentrations of the viruses of lymphogranuloma venereum[139]; equine encephalomyelitis[143] and epidemic keratoconjunctivitis[140] at room temperatures than at 37°C. Hecke[64] observed satisfactory multiplication of the virus of foot and mouth disease at 30°C., and Parker and Hollender[105] have fixed the optimal temperature for the growth of Theiler's mouse encephalomyelitis virus at 35°C. In a study of the effect of temperature on the multiplication of the yellow fever virus, Fox[50] found the optimal range to be between 32°C. and 37°C., although growth proceeded within the extremes 27°C. to 39.5°C. In the evaluation of such data one must bear in mind that changes in temperature may affect the metabolic activity of the tissues as well as the rates of increase and inactivation of the virus.

Illustrative Examples of the Behavior of Viruses in Tissue Culture. A brief summary of the characteristics exhibited in tissue culture by a few selected viruses will serve to integrate certain of the materials that have been discussed so far and will also illustrate the wide differences in behavior of

individual viruses—phenomena that we consider worthy of much emphasis.

Vaccinia. It has been found possible to propagate this virus in the simplest type of tissue culture consisting of chick embryo tissue suspended in Tyrode's solution. Under the proper conditions, inclusion bodies are formed in the tissues and the development of these bodies may be used as an indication of growth of the virus.

Curves illustrating its multiplication as reflected by the concentration of virus in the tissues and fluid phase of roller tube cultures of chick embryonic tissues are presented in Fig. 2. In considering these data it should be kept in mind that the fluid phase was replaced every 2 days. A rapid increase in the virus of both phases is followed by a long period during which there is little change in its concentration. The finding of a greater quantity of virus in the tissues is characteristic of most of the agents that have been investigated from this point of view. These include psittacosis[171] and yellow fever[50]. Sanders and Jungeblut[142] on the other hand found that the concentration of Columbia SK virus was greater in the fluid portion of the culture than in the tissues.

We have noted previously that vaccinia virus does not cause extensive damage of the tissue in which it is propagated. After prolonged contact, however, under suitable conditions there is some evidence that this agent is capable of injuring the cells.

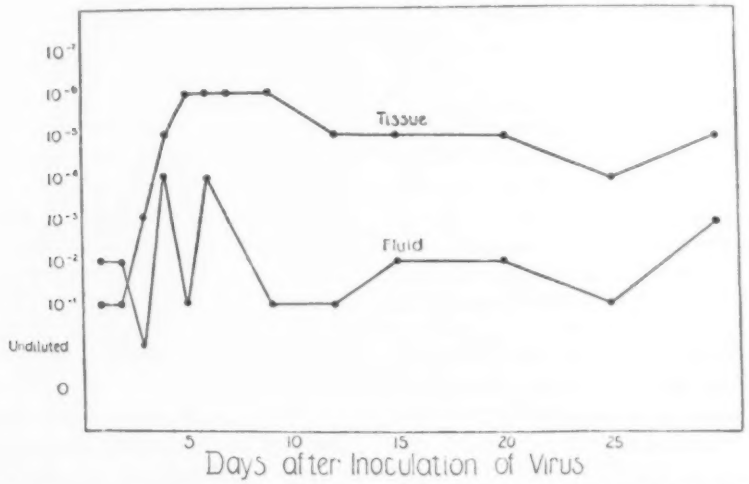

FIG. 2.—Quantitative estimations of vaccinia virus in the tissue and fluid of individual cultures selected at various intervals from a series of similar cultures of cardiac muscle obtained from 11 day chick embryos. Ordinates indicate highest dilution of the materials giving a definite reaction in the rabbit's skin. (Reprinted from J. Exp. Med., **72**, 367, 1940. Feller, A. E., Enders, J. F., and Weller, T. H.)

Mumps and Influenza. The viruses of mumps and influenza exhibit similarities as well as some interesting differences. Both are readily propagated in suspended cell cultures of chick embryonic amniotic or allantoic membrane and both show a certain selectivity as to other tissues in which they will grow. In roller tube cultures Pearson and Enders[108] found that the virus of influenza grew well in various chick embryonic tissues including the membranes, lungs, intestine and skin, but

poorly in brain, skeletal muscle and heart. In suspended cell cultures, on the other hand, chick embryo brain appears to support the growth of influenza virus quite adequately[164]. The mumps virus which grows well in chick embryo cultures multiplies much less actively in cultures of human embryonic parotid gland and pancreas[84]. Another important similarity between the viruses of mumps and influenza is the production of hemagglutinin in tissue culture[164]. Neither virus appears to exert any harmful effect upon the tissue in which it is propagated.

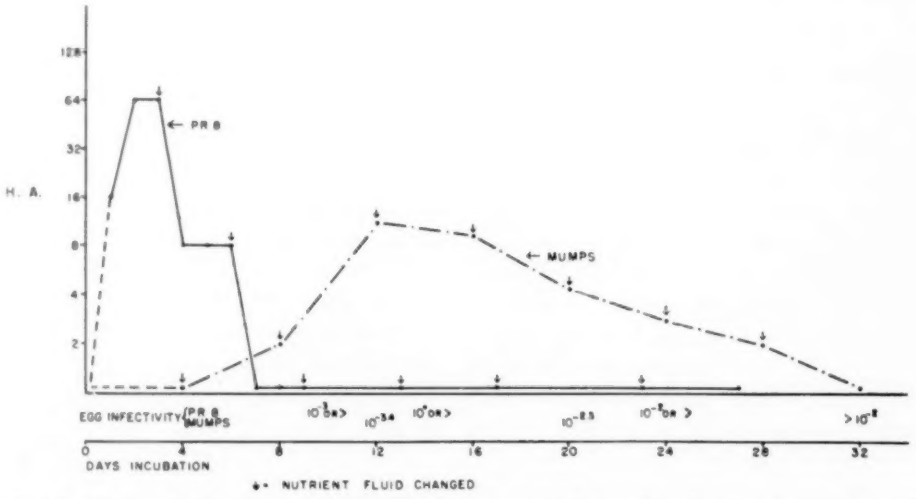

FIG. 3.—Hemagglutinin and infectivity titers in suspended cell cultures of mumps and influenza virus (PR 8).

A striking difference in the behavior of these 2 viruses is evident when one compares their growth curve in suspended cell cultures of chick embryo amniotic membrane. As shown in Fig. 3, the mumps virus increases relatively slowly, usually reaching maximum concentration in the fluid phase on about the 12th day of cultivation. Virus is still developed over a period of a month or longer with a gradual decline in its rate of production. In contrast, the virus of influenza increases rapidly, attaining a peak at 48 to 72 hours. Thereafter relatively little additional virus is produced. The cause of this abrupt decrease is as yet unknown. It is probably not due to injury of the tissue, since the cells of a culture previously infected with this agent are still capable of supporting the growth of the virus of herpes simplex. It is possible that the influenza virus rapidly exhausts some factor essential for its active growth but not essential for the metabolism of the cells.

Lansing Poliomyelitis. The Lansing strain of poliomyelitis virus has been found to multiply in suspended cell cultures of various human embryonic tissues as well as in cultures of prepucial tissue from young children[166]. As already mentioned no evidence of multiplication has been obtained in similar cultures of chick or mouse embryonic tissue or adult mouse brain.

In cultures of human embryonic skin and muscle infected with a relatively small quantity of Lansing virus an increasing concentration in the fluid phase occurs during a period of about

20 days. Thereafter a rapid diminution of virus production ensues.

As we have lately found, this virus injures the tissues after a period of contact of from 8 to 20 days. It is of interest to observe that most of the various cell types in fragments of skin and muscle or intestine appear to be affected. This finding was unexpected since this agent has been regarded as a strict neurotrope.

Equine Encephalitis. In contrast to the virus of poliomyelitis those of Eastern and Western Equine Encephalitis increase with great rapidity in cultures and promptly destroy the cells. Cell death is closely followed by the disappearance of virus from the system.

It is apparent that the viruses that we have just mentioned as representing various types of behavior may be arranged in a series according to their cytopathogenic effect. Those of mumps and influenza seem to be almost incapable of injuring the cells in which they multiply. Vaccinia and Lansing poliomyelitis viruses damage cells only after relatively long periods of contact. The equine encephalitis viruses destroy them with extraordinary rapidity.

Application of Tissue Culture Techniques. The most significant achievement of the tissue culture method in the field of viruses is the development of the attenuated strain of yellow fever employed for vaccination.

Haagen and Theiler[60] demonstrated that a highly virulent pantropic strain of this virus could be adapted to cultivation in chick embryonic tissue. In this medium the virus became modified[88,152], so that it was no longer pathogenic for monkeys or man but retained its antigenicity. This modified virus has been used as a living vaccine and has served as an important factor in the successful control of yellow fever throughout the world.

Like that of yellow fever, certain other viruses have become modified after propagation *in vitro*. Koprowski and Lennette[79,80] have reported that following prolonged maintenance under these conditions the Venezuelan equine encephalitis and West Nile viruses showed a reduced capacity to infect mice by the intraperitoneal and subcutaneous routes whereas no change in their infectivity was observed when they were inoculated by the intracerebral route. Similar behavior has been described in the case of the Columbia SK virus[142]. The virus of vaccinia appeared to lose its antigenicity after long periods of cultivation[131]. Parker[107] reported that one strain of this virus that had been maintained for years in tissue culture became relatively avirulent for rabbits but retained its ability in infect the chorioallantoic membrane of the chick embryo.

The viruses of Eastern and Western equine encephalitis and vesicular stomatitis virus have been maintained for long periods in cultures of chick embryonic tissue without undergoing significant change in their pathogenic properties[100]. Yanamura and Meyer[171] found no loss of virulence in psittacosis virus that had been maintained in culture of chick embryonic tissue for 2½ years and Plotz[113] demonstrated only a slight drop in the concentration of fowl plague virus present in cultures of chick embryo tissue after five years of cultivation.

With the exception of vaccine against yellow fever, the method of tissue culture has not been widely used for the preparation of prophylactic agents. However, Rivers and his co-workers[131, 132] and Plotz[116] showed that vaccinia virus maintained in tissue culture could be used as a source of virus for the vaccination of man. Vaccines effective in

experimental animals have been prepared from tissue culture preparations of psittacosis[99], influenza[51], and rabies[161].

In the study of the problems of immunity, numerous investigators have found tissue culture techniques of value[3,4,5,49,128,157]. Much of the available information about the role of the cell in immunity to viruses has been obtained in this manner[39].

Tissue culture techniques have yielded information concerning the morphological development of certain viruses. This is illustrated by the work of Bedson and Bland[11] and Bland and Canti[14] with psittacosis; Gey and Bang[53] with the virus of lymphogranuloma venereum, and Bland and Robinow[15] with the virus of vaccinia. More recently, interesting data concerning the morphology of certain viruses and virus-like particles and their relation to the cell have been acquired by means of special tissue culture techniques which make visible the interior of cells by means of the electron microscope[7,17, 121,169].

The work of Thompson[153] and Pearson[109] mentioned above illustrate the application of *in vitro* methods to the investigation of the metabolic requirements of viruses. Early and Morgan[37], on the other hand, explored the effect of certain chemotherapeutic agents upon psittacosis in tissue cultures. Andrewes[6] as well as Lennette and Koprowski[83] studied in tissue cultures the problem of the interference phenomenon; that is, the inhibitory effect that one virus may exert upon the growth of another.

Some of the most ingenious applications of a practical nature that have been made of tissue culture techniques are those of Huang[71,72]. Using the fact that the virus of equine encephalitis rapidly reduced the ability of the infected tissue to proliferate and to metabolize glucose as an index of the presence of the virus, he was able to perform titrations and even neutralization tests *in vitro* without using experimental animals. Working with the viruses of influenza Magill and Francis[93] in 1938 had performed neutralization tests in suspended cell cultures, as part of their study concerning the antigenic strains of this virus. However, in contrast to Huang they used animal inoculation as a means of indicating the presence of the virus.

Huang[70] also found it possible to do titrations of St. Louis encephalitis and Columbia SK viruses using as evidence of the presence of these agents the fact that cultures infected with either one would not support the growth of equine virus if it was added several days later. The St. Louis encephalitis and Columbia SK viruses did not in themselves visibly injure the tissue.

The use of tissue cell cultures for the primary isolation of viruses from infected animals and man has been described. Cox[26] reported that suspended cell cultures of chick embryo tissues were more sensitive in detecting the presence of equine encephalitis viruses than were either mice or guinea pigs. Sanders and Huang[141] described small tube cultures that they felt could be used for isolation of viruses in field investigation. By the use of sulfonamides they were able to isolate viruses from sputum and mosquitoes in such cultures. We[133] have recently been successful in isolating poliomyelitis virus from stools of 2 patients by direct inoculation into tissue culture of stool suspension to which streptomycin and penicillin had been added. Sanders and Alexander[140] found that the virus of epidemic keratoconjunctivitis could be more easily isolated from patients in tissue culture than by inoculation of mice. Only after the agent had been passed through tissue culture did it

regularly produce a disease in these rodents.

Suggestions for Further Investigation of Viruses by Means of Tissue Culture Techniques. Although the developing chick embryo provides a more convenient medium for the routine propagation of many viruses, for certain agents and for special purposes the tissue culture appears to offer advantages over the living animal, including the chick embryo. These are dependent upon the following facts: 1, tissue cultures represent a great simplification of the conditions under which the virus multiplies as contrasted with the living animal; 2, they offer conditions under which cultivation of the virus may be more easily and accurately controlled; 3, they afford the means of employing tissues of susceptible hosts such as the human being which are not readily available for inoculation with the agent.

With the foregoing statements in mind, it may be profitable in conclusion to suggest some lines of investigation which may be pursued in the future. These in turn may be grouped under 3 main headings: *a*, studies on the growth of viruses; *b*, studies on the cytopathogenic properties of viruses; *c*, studies on the factors involved in natural and acquired resistance to viruses.

Although much has already been learned in respect to the broader aspect of the growth behavior of viruses, there remains much to be discovered as to the underlying mechanisms. For example, we do not know whether the majority of mammalian viruses increase in a step-like manner comparable to that established for bacteriophages. Using the chick embryo, evidence has been lately presented that the growth of influenza virus presumably proceeds in this way. To the authors it would seem that such analyses might be carried out with greater accuracy in tissue cultures where the number of cells available can be estimated more accurately.

The elucidation of the conditions responsible for the self-limiting character of the growth of influenza as contrasted with those of mumps and vaccinia would appear to render information of fundamental interest and possibly practical value. It would appear that an approach to this problem could be best made through the techniques of tissue culture.

The attempt to identify substances of known composition which may act as growth factors for viruses is in its infancy. Clearly, progress will be most rapid in this field if the relatively simple system of the tissue culture is actively exploited. Similarly, in the further search for drugs and antibiotics that may exert viricidal or viristatic effects the method should prove useful; especially in the case of those agents which are pathogenic only for man or for man and the primates and which can be grown *in vitro*.

For viruses that so far have been found strictly pathogenic for man, cultures of human tissues may furnish the only method by which they can be isolated and then studied in the laboratory. A number of agents of this class still remain such as the viruses of infectious hepatitis, chicken pox, infectious mononucleosis, rubella and probably measles.

As we have pointed out, preliminary successes have been recorded in the primary isolation of viruses in tissue culture. The practicability of the method for this purpose deserves much further investigation; since in certain diseases it might prove a valuable diagnostic aid both from the clinical and epidemiologic points of view.

The mass cultivation of viruses *in vitro* for the purpose of obtaining antigens to be used in diagnostic tests or

for vaccines seems at first sight to be of little practical value for most viruses, since other methods are more convenient and often produce higher concentration of the agent. But future research may reveal ways whereby yields of virus-containing material may be obtained which might lead to the adoption of the method for these purposes. It is, indeed, evident at the present time that considerable quantities of certain viruses may be easily produced and these in a menstruum relatively free of contamination by molecules of large size. In this latter respect at least, tissue culture virus would seem to provide a suitable material for the preparation of vaccines for experimental work or as starting material in procedures directed toward the purification of the agent.

The details of the manner in which viruses give rise to pathologic changes are still largely obscure. The way to new procedures for the prevention and therapy of viral infections might well be indicated if the mechanism of the injury induced by these agents were more completely understood. It is, therefore, important to maintain intensive research on the following problems. First to determine how the virus particle gains entrance to the susceptible cell—a problem that in spite of much study still remains unsolved. Conversely it would be useful to be able to recognize the factors which prevent the invasion of cells which are naturally resistant. It may be anticipated that cultures of pure lines of cells would be of aid in such investigations.

Once the virus has entered the cell it is possible to visualize several processes by which it may cause injury and cell death. Thus its continued proliferation may give rise to internal pressure sufficient to rupture the cell membrane. In this way, it has been shown, bacteriophage destroys its host and, in the case of psittacosis, and vaccinia virus, evidence obtained from studies in tissue culture and by other techniques suggest that the same mechanism may be, in part at least, responsible. Additional researches of this sort aided by the electron microscope might well be fruitful.

During the past few years it has been demonstrated that, in large quantities, viruses like influenza[68] and those of the lymphogranuloma venereum-psittacosis group[123] are toxic for animals as indicated by rapid collapse and death following their inoculation. We have no information, however, as to the manner in which the toxin produces these effects. Is it on the cells in which the viruses naturally multiply or is on those of the vascular or nervous system? Again we may believe that the tissue culture might prove a useful tool in the solution of such problems.

It is logical to think that the normal metabolism of the cell is deranged to a greater or less degree during the phase of virus increase, and before cell-death, if this occurs at all, has yet taken place. Evidence for this hypothesis might be obtained by further careful studies of the O_2 consumption in tissue cultures infected with a variety of agents according to the method of Zinsser and Schoenbach[173], as well as by the application of histochemical techniques now available for testing for the different types of nucleic acids and other biologically important substances.

The conditions that induce mutations in the pathogenic characteristics of virus are known only in a most general way. That such changes may occur spontaneously in tissue cultures we have already noted. To define these conditions more precisely and to be able to establish them at will would be a highly significant achievement. The progress that has been made of late in the experimental production of mu-

tants among certain species of fungi and bacteria would appear to warrant the application of the same procedures to animal viruses undergoing multiplication *in vitro* in the expectation that useful mutant forms might emerge.

It is worthy of remark that few studies of the effect of tumor producing viruses on cells growing *in vitro* have been made since Carrel published his original observations on the Rous sarcoma virus. Certain objectives for further researches are obvious. For example, it would be important to undertake experiments to determine whether virus could be eliminated from cells which it had apparently rendered malignant. If means could be found to accomplish this, it would be of great theoretical interest to know whether or not such cells still retained the capacity to initiate tumors.

It has been pointed out that a good deal has been learned concerning the natural resistance of different types of cells and of cells of different species of animals by means of experiments with tissue cultures. Moreover, basic observations concerning the predominating role of humoral antibodies in acquired immunity to virus infections have been made in a similar manner. It is likely, however, that additional knowledge can be gained concerning the mechanisms of natural and acquired resistance by application of the method. An answer might be sought to such a fundamental question as to whether or not antibody globulin can under any circumstances pass the cell membrane, and if it does so, whether it can there inhibit the multiplication of virus. The available evidence strongly suggests that the answer is in the negative; but this evidence is not entirely conclusive. Possibly by invoking the aid of the technique recently described by Coons and his associates[25], by means of which antigenic substances may be demonstrated with precision in tissues, decisive results might be obtained.

In conclusion, we hope that this review of some of the contributions of the tissue culture method to our understanding of the behavior of viruses may serve to emphasize the fact that its usefulness has by no means been exhausted. Indeed we believe that as its techniques are developed further and become increasingly more quantitative through the application of modern physico-chemical and biochemical knowledge, they will contribute substantially to the solution of the final problems of the true nature of these agents and of the effective control of the diseases they incite.

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PREVENTIVE MEDICINE AND EPIDEMIOLOGY

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EPIDEMIC DIARRHEA OF THE NEWBORN

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RECOGNITION that a new infectious disease is present in a community is today something of an event. To find that it is well established within areas as large as those defined by national boundaries and that it constitutes a continuing problem is still more unusual. Yet that is the situation with epidemic diarrhea of the newborn.

The activities set in motion by the presence of a new disease tend to follow a common pattern. The condition is studied clinically, by methods of the experimental laboratory, and epidemiologically[42], by all of the methods medicine has for acquiring new knowledge. The information that follows is sometimes as satisfactory as that for rickettsialpox[46]; at other times it is all too meager, as with infectious diarrhea of the newborn. A program of prevention and control ultimately is evolved, sometimes ordered and precise[6] as for rickettsialpox, sometimes wholly empirical as for epidemic diarrhea of the newborn. In varying degree incidence decreases, interest lags, and attention is transferred to some other disease or awaits a fresh wave of epidemics. Thirteen years have passed since epidemic diarrhea first came seriously to attention, and it is still a problem of nursery populations. The purpose here is to determine what has been learned in that period and what remains to be learned, to review accomplishment in control of epidemics, and to suggest the direction of effort toward a more adequate prevention.

THE FIELD OF INTEREST. Infant mortality rates in the United States have declined steadily since 1900, but the improvement in neonatal deaths is less marked. In New York City[36c] death rates for infants aged 1 month to 1 year were 35.5 per 1000 live births in 1926, and 12.3 in 1939. The corresponding rates for infants less than 1 month old were 32.2 and 24.8. Much of the reduction in neonatal mortality is in respect to congenital defects, birth injuries and

prematurity. Deaths from acute diarrheal disease during the 1st month of life actually increased, at a time when deaths from that cause were less for infants aged 1 month to 1 year. The differences are suggestively greater than indicated by reported demographic data. In our experience there have been repeated instances where infectious diarrhea of the newborn was disregarded as a cause of death, even during well-defined outbreaks, and recorded as prematurity or bronchopneumonia.

Acute infections of the intestinal tract are, of course, an old story among causes of death in infancy, from the days of cholera morbis and long before that. As microbiologic methods were introduced into clinical practice, many cases were identified as due to known pathogens, principally of the salmonella and shigella groups. Well-defined epidemics were traced to the same microorganisms. Always, there remained an imposing bulk of cases and of epidemics in which no one of the accepted pathogens of man could be identified. About 1930, reports[31,32,48] began to appear, suggesting the existence of an individual disease, with a predilection for infants in the first days of life and largely limited to infants aggregated in nurseries. The problem was brought to a focus in this country in 1937 with the publication of a series of reports by Rice, Best, Frant and Abramson[2,36a,36b,70] which recognized the disease as epidemic diarrhea of the newborn. The course of events in Great Britain was much the same. Although death reports of 1930 to 1938 give little specific evidence of this form of neonatal enteritis, epidemiologic analyses[82] showed that a significant proportion of deaths were associated with one another in time and place.

As a point of reference, to place epidemic diarrhea of the newborn among allied conditions, the recently issued *Manual of the International Statistical Classification of Diseases, Injuries, and Causes of Death*[81] is taken as a guide to the acute diarrheal disorders of infants and children. A group of specific infections caused by salmonellae is first set apart, including typhoid fever, the paratyphoid fevers of types A, B, and C, and a 3rd division for other salmonella infections, further subdivided according to whether or not food is mentioned as a vehicle. Bacillary dysentery forms the 2nd general group, with distinction according to etiologic agent as Flexner-Boyd, Schmitz, Shiga, Sonne, and others, and unspecified. Except for the more chronic infections caused by amebae and other intestinal parasites, these are the known specific infections of the gastrointestinal tract. Further classification is by clinical and epidemiologic criteria. Food poisoning, to include both infections and intoxications, has independent status. The remaining classes are by age and symptomatology. Diarrhea of the newborn (Rubric 764) includes all deaths and cases of gastroenteritis and colitis at any age when specified as diarrhea of the newborn. For classification of deaths, it includes all fatalities from gastroenteritis and colitis at ages under 4 weeks. In morbidity classification, it includes all cases, lethal or non-lethal with onset at ages under 4 weeks. Rubric 571 is devoted to gastroenteritis and colitis, except ulcerative, at over 4 weeks; and to diarrhea of unspecified cause at 4 weeks to 2 years. Diarrhea at ages 2 years and over falls into classification 785.6. Discussion is here limited to the diarrheal condition defined as epidemic diarrhea of the newborn (Rubric 764). Infections with known pathogens of the salmonella or shigella groups are excluded.

The Clinical Disease. In the absence of an outbreak, recognition of epidemic diarrhea of the newborn is not possible. Even with 2 or 3 cases

in a nursery, the clinical suggestion may be of other conditions associated with diarrhea, parenteral or non-infectious. As a consequence, authorities[20] recommend adequate provision of isolation units and prompt separation of questionable and suspicious cases until their nature is determined.

The onset is sometimes insidious, but more commonly abrupt. Vague premonitory indications of gastrointestinal disturbance sometimes precede by 24 or even 48 hours the severe symptoms of the fully developed disease. Some patients are indifferent to food and fail to gain weight. Others are drowsy, listless, or experience distension and occasional vomiting. Profuse diarrhea and vomiting are characteristic of sudden onset. As diarrhea persists, the infant becomes dehydrated and has a dramatic loss in weight, as much as 1 lb. a day. Stools are frequent, watery and yellow, later changing to green. Blood, pus, mucus or curds are unusual. Stools are expelled forcibly and with increased frequency. The buttocks become red and excoriated. Fever is not a prominent feature and temperatures ordinarily remain normal or only slightly elevated until the terminal rise so commonly associated with bronchopneumonia.

The clinical course of severe infections is unmistakable. The expression is anxious and pinched, and the skin extremely dry. Color is ashen gray. As dehydration and acidosis become aggravated, the fontanelles and eyeballs are sunken. Drowsiness deepens into coma, and the cry is feeble and short. At this stage, vomiting is severe and diarrhea profuse. Urinary output decreases and there is marked abdominal distension. Except for redness of the throat, significant physical findings are absent.

Death may occur in 24 hours or be deferred several days. Total days of illness for 25 fatal cases varied from 2 to 29, with an average of 8[73]. As with most acute infections, the number of fatal cases tends to be greatest at the beginning of an outbreak, but this is not always so. Mild infections may continue for several weeks before the first fatality. Recovery tends to be slow, and marked by decrease in diarrhea and arrest of weight loss. The course is generally uncomplicated. Recurrence of diarrhea after a quiescent period has been noted. Otitis media is fairly common, suppurative lesions of the skin[58b] occur, and death from hemolytic staphyloccocal pneumonia may be the terminal event.

Pathologic Anatomy. Post-mortem examinations have revealed nothing in the way of pathognomonic changes permitting earlier recognition or a better understanding of the basic nature of the disease. Pneumonia, lung abscesses and hyperemic spots on the intestinal mucosa have been described[9]. Craig[25] found hyperemia and swelling of the intestinal mucosa, with some involvement of Peyer's patches and the mesenteric lymph nodes. Two patients had early leptomeningitis, and 2 others marked intestinal ulceration. Similar observations were reported by Greenberg and Wronker[43]. Others[56] have reported edema of the brain and marked erosion of the intestinal mucosa with multiple minute submucosal hemorrhages. The universal observation is a lack of anatomic change commensurate with the severity of clinical manifestations. The usual case shows a minimal amount of hemorrhage and ulceration of the intestinal tract, in contrast for example to the unmistakable lesions associated with bacillary dysentery.

Differentiation of Other Epidemic Diseases. Clinical diagnosis is commonly by exclusion and by failure to demonstrate one of the pathogenic bacteria responsible for acute diarrheal

infections of infants. Other acute infections that produce manifest epidemics among newborn infants, therefore need consideration.

Bacillary dysentery among the newborn is rare. Of a goodly collection of epidemics at our disposal, more than 40, none was of that nature. Search of the literature reveals only 3[5,49]. Salmonellosis is somewhat more frequent. Differentiation of both on clinical grounds may be difficult, but bacteriologic examination of the blood and stools has much value. Fever is a regular accompaniment, and the clinical course is frequently marked by bacteremia and sepsis, with signs and symptoms of pneumonia or otitis media. Aballi *et al.*[1], and Abramson, Frant and Oldenbusch[3] have reported salmonella outbreaks among the newborn in which meningitis was the principal manifestation.

Epidemiologic Attributes. The incubation period is indefinite. Information derived from the course of an outbreak, having the purpose of measuring the interval between the initial and subsequent cases, is without much value. Odd groupings occur, the distribution of cases within a nursery is most irregular, and the usual result is complete inability to trace infection from case to case and sometimes to eliminate the possibility of a single mass exposure. Study of the secondary case thus lacks its usual usefulness. Infections have been recognized on the 2nd and 3rd days of life[11,54], which suggests a short incubation period. The usual experience is 6 or 7 days, with stated limits between 2 and 21 days[6]. Babies are known to have been exposed to the infection in hospitals and to have been discharged, only to develop symptoms as long as 20 days thereafter. From what is known of the behavior of the disease, a subsequent exposure is the more likely explanation.

Nursery outbreaks have been recorded in Massachusetts[73] and in New York City[70] at all seasons of the year. When outbreaks were collected according to the season in which they started[36c], average attack rates were greater in spring and summer than in autumn and winter, with spring and summer showing no material difference.

Individual outbreaks are frequently of long duration. Ormiston[67] described 3 that extended respectively from May 2 to July 20, June 17 to December 15, and July 2 to September. That of Brown, Crawford and Stent[14] lasted from May to August. Outbreaks characterized by 2 epidemic waves[53] are not unusual, with the first wave extending, for example, from January through March and the second from April through July. Such outbreaks[17] have in other instances been interpreted as independent phenomena brought about by a 2nd introduction of infection. The observed persistence of infection, once established in a nursery, and the long course that many epidemics follow, make cyclic variation within a single outbreak the more tenable explanation.

Some outbreaks are characterized by an appreciable series of mild infections before severe and fatal cases occur. In one instance the elapsed time between the initial case and the first death was 29 days, in 2 others 13 and 20 days. These epidemic attributes of long duration and slow evolution have the effect of inhibiting recognition of the process by hospital authorities and consequently delayed notification of health departments. Of 19 outbreaks recorded in Massachusetts in a 10-year survey[73], only 6 were reported within 7 days of the 1st case. In 10 instances, a month elapsed before notification, while in 3 outbreaks the interval was 50 or more days between the first case and first

report. Many infants were unnecessarily exposed.

Epidemic diarrhea of the newborn is a truly epidemic disease. Attack rates are high and deaths numerous[36b], although, as with all communicable processes, epidemic behavior is subject to variation. A comprehensive study of 40 outbreaks[58b,73] in nursery populations showed a range of morbidity from 10 to 60%. Case fatality varied from nothing to 88%. Based on the same experience, average attack rates are about 24% and the expectancy for deaths per 100 cases is in the neighborhood of 33.

The sources of infection are unknown. Such evidence as exists supports the reasonable assumption that the reservoir is man, and that the mode of transmission is direct or indirect person-to-person infection. The difficulty lies in lack of information as to whether infection is limited to the age group concerned or is more widely distributed in the population, perhaps manifesting itself in other clinical forms or as inapparent infection by reason of acquired resistance. At a time when diarrhea of unknown etiology was widely prevalent in Boston, spread of infection occurred from patients of the pediatric wards of a hospital to newborn babies in the hospital nursery. Both groups had deaths, the fatality for nursery infants being 27%, and that for older children of the pediatric ward 15%. A survey of hospital populations throughout the metropolitan area revealed concurrent infectious diarrhea among the newborn in 5 different hospitals. Symptomatology, morbidity and fatality were in agreement with the classical disease. Concurrent outbreaks in nurseries of a community have also been observed in Australia[17], with 3 outbreaks in 2 hospitals from early May to August, 1943. Except for a nurse with vague gastrointestinal disturbance just preceding one nursery outbreak, no intestinal illness was discovered among mothers or nursery attendants. Coincident outbreaks in the hospitals of a community is an epidemiologic feature not sufficiently emphasized.

Cumming[28] reported a nursery outbreak among infants where several mothers were then convalescent from a diarrhea generally prevalent throughout the Washington, D. C., area. Another outbreak[4] spread from adults on a maternity service to infants of the nursery and subsequently to children in an institution and others in private homes. An outbreak termed "gastroenteritis of unknown etiology"[23] started in the maternity unit of a hospital in Oxford, England. Mothers commonly became ill 5 to 9 days after admission. Babies, patients, hospital staff and household contacts were eventually affected. Where both mother and child were ill, infection of the infant preceded illness of the mother in 22 instances, in 15 cases was later, and coincided 5 times. Secondary cases in the home followed discharge of patients from the hospital. The circumstance loses some significance in that the clinical manifestations were less severe than with epidemic diarrhea of the newborn as seen in the United States, but the disease is recognized as being generally milder in Great Britain[23,37].

With this understanding of the clinical nature of the disease, of the pathological changes it brings, and of its mass behavior in epidemics, attention is now directed to the specific features of host, agent and environment in a search for causes of outbreaks.

The Host Factor. Opinion about the age distribution of epidemic diarrhea of the newborn is undergoing change. Earlier descriptions emphasized a limitation to the neonatal pe-

riod, with minimal or no involvement of older infants, children or adults[70]. In the outbreak of Buddingh and Dodd[15], spread of infection took place from adult attendants to infants and extended to household contacts 6 and 8 years of age. The mildness of the disease and the presence of stomatitis suggest an etiological agent not usually associated with epidemic diarrhea of the newborn. In other outbreaks[58b] conforming wholly to accepted clinical interpretations, infants beyond the neonatal age and older children have also developed symptoms indistinguishable from epidemic diarrhea of the newborn. Six outbreaks in hospitals of San Francisco[38] gave an age distribution for 45 fatal cases of 33 patients less than 1 month old, and 12 past that age, of which 4 were aged 2 months or more. One last practical consideration bearing on age is that the longer newborn infants are held in hospitals, the greater is the likelihood of contracting the disease. Presumably, this is more a matter of increased opportunity for exposure than an optimal susceptibility during the later neonatal period.

Outbreaks of a generally similar disease and of demonstrated viral origin are no uncommon occurrence in institutions for adults[40]. No relationship between the two has been established, either through comparison of agents isolated, by immunity reactions, or epidemiologically.

In the Massachusetts experience[73] both morbidity and case fatality were greater for premature infants than for those born at term. The comparative morbidity rates of 49 and 21%, and fatality rates of 53 and 25% indicate both a greater susceptibility of premature infants and a lesser ability to cope with the infection.

The evidence of protection against epidemic diarrhea of the newborn through breast feeding is conflicting. In the outbreaks reported by Lembcke, *et al.*[54], 54% of 33 infants fed by formula became ill, compared with 21% of 144 breast fed babies, the differences being significant. Mayes[59] also reported that breast fed infants fared better. Campbell[17] by contrast found the majority of her cases were among breast-fed infants; and Frant and Abramson[70] found no differences in attack rate between the 2 groups. In Massachusetts, outbreaks have involved both artificially and breast fed infants. Since breast fed babies shortly after birth commonly receive either supplementary feeding by formula or lactose solution, the distinction between the 2 methods of feeding is not always complete. That protection is afforded through transmission of maternal antibodies by milk would seem to have little support.

The Infectious Agent. The agent of disease in epidemic diarrhea of the newborn is unknown. It is clear that no one of the common pathogenic bacteria of the human intestinal tract is responsible. The bacteriologic findings are most diverse, from epidemic to epidemic and frequently within the same epidemic. That both shigella and salmonella have given rise to epidemics of diarrhea in nurseries is clearly evident. Various types of salmonella, to include S. *wichita*[62,75], S. *typhimurium*[74,79], S. *senftenberg*[74] and S. *choleraesuis*[74], have been involved somewhat more frequently than shigella[5,49,79]. The spread of infection was characteristic of the agents concerned, with a mother identified in several instances as the source of the outbreak. The critical consideration is that of the hundreds of outbreaks studied, these known agents of intestinal infection have been isolated in relatively few. With facilities for bacteriologic examination generally available and with these microorganisms so readily demonstrable, the conclusion is reasonable that if this evidently

communicable disease is a specific infectious process, then some other agent is responsible.

The first requirement for a microorganism to be considered as the cause of an outbreak is that it be found with regularity and in appreciable numbers in most cases of the epidemic. In a nursery outbreak involving infants of ages 6 weeks to 10 months rather than the newborn, Walcher[78] recovered *B. mucosus capsulatus* from 22 of 24 patients. The stools of all were free from the microorganism one month later. Only 2 of 107 healthy infants carried the organism. Jampolis *et al.*[48] in 1932 had reported similar findings among newborn infants, and additionally, the presence of *B. mucosus capsulatus* in the throats of 3 adult contacts. Single bacterial agents of other nature have also predominated among cases of diarrhea in newborn infants. Hemolytic streptococci[26] were recovered from the throats of 14 sick infants, the blood of 2 others and from the stools of all 16. Cass[18] isolated *Aerobacter aerogenes (B. lactis aerogenes)* from the feces of 5 sick infants. During a community-wide outbreak of diarrhea, with milk the likely vehicle, Ensign and Hunter[34] recovered *Pseudomonas aeruginosa* from patients of a hospital nursery. Nine died among 24 affected.

Most of the bacteria found to predominate among patients of an epidemic are not accepted pathogens for adult humans. No one has been demonstrated with any regularity. Different species are found in outbreaks which are strikingly similar, clinically and epidemiologically. Their presence would seem to depend more on the opportune features of the environment than on a particular biologic characteristic of the microorganism that gave unusual capacity to produce disease.

A mixed flora, with some potential pathogen present, has characterized bacteriologic study of stools in other epidemics. Much variation from patient to patient within the same epidemic is usual. Staphylococci were isolated in outbreaks reported by Dulaney and Michelson[31], Baker[8], and Felsen and Wolarsky[35]. *E. coli* was the suspected causative agent in several outbreaks[12,25,27,60]. Others include monilia[32,66], *B. proteus vulgaris*[24] and *clostridium welchii*[51]. The marked changes that take place in the bacterial flora of healthy newborn infants, in the transition to what becomes accepted as the normal state for older children and adults, are not always appreciated. Kneeland[52] has described the alterations in nasopharyngeal flora during early infancy. The changing pattern of the intestinal flora has been widely studied[45,77]. Differences between breast fed and artificially fed infants are well known[39]. It is therefore not surprising that bacteriologic studies during the course of intestinal outbreaks at these ages show much variation. Examination of stools of normal infants under similar environmental conditions, and concurrently with the course of an epidemic would be informative.

The lack of uniformity among bacterial findings naturally directed effort toward other microbial forms, and to a virus or a group of viruses. Ether-treated stools[54] from infants ill with epidemic diarrhea of the newborn were inoculated intraperitoneally into baby Swiss mice. Specimens from patients killed 39% of mice; from normal controls, 11%. The interpretation was that some agent lethal for mice is frequently present in feces of infants ill with infectious diarrhea of the neonatal period.

Buddingh and Dodd[15] studied a nursery outbreak among the newborn in which diarrhea was a prominent symptom, but which was atypical in that most patients continued to take

feedings and gained weight. Transfer of swabbings from the tongue and gums and from stools to the cornea of rabbits led to a characteristic inflammatory reaction. Swabs from normal infants were without result. Reactions were obtained with materials from adults with sporadic stomatitis, and also from adults with no clinical evidence of infection. Three of 4 nurses with duties related to the nursery gave evidence of the presence of virus, one having lesions of stomatitis. One of the other 2 nurses developed stomatitis the week following the outbreak. Her own 2 children, aged 6 and 8 years, in turn had lesions of the mouth, confirmed as of similar nature by rabbit inoculation. The nurse-mother still carried virus 3 months later. Meiklejohn[63] reported similar findings.

The observations of Light and Hodes[55a] first reported in 1943 are most suggestive. Four nursery outbreaks were studied, 2 with high morbidity and 2 with the usual morbidity but a fatality that was respectively low and moderate. Suspensions of stools from patients were introduced nasally into calves. Bloody mucoid diarrhea followed. Filtrability of the agent was established for each of 4 strains isolated from the separate epidemics. All have been passed serially through calves[55b]. Exposure of the agent to heat at 80°C. for 1 hour led in 1 instance to inactivation and in another to attenuation.

The demonstration of MG streptococcal antibodies and of cold agglutinins in serums of infants recovered from acute diarrhea has recently been advanced as further suggestion of viral causation in this group of diseases[50]. The patients were older infants, usually in the 1st year of life and with no suggestion of primary atypical pneumonia.

THE ENVIRONMENT. A brief experience with outbreaks of epidemic diarrhea of the newborn will demonstrate the importance of physical and social factors of the environment in causality. There is the matter of feeding. Bacteriological examination of nipples and prepared feedings in nurseries where an outbreak is present commonly reveal[53] large numbers of bacteria, among them staphylococci, *B. subtilis*, and gram-negative bacilli. Field study of nursing techniques and of methods of preparing formulas[73] in our experience has repeatedly demonstrated deficiencies, often in direct violation of state regulations, and easily sufficient to account for observed bacteriologic inadequacies. Bacteria judged identical by accepted methods were recovered from infants, from materials used in their care and from milk mixtures with which they were fed. In one instance, Group D streptococci were isolated from the throats of 9 infants, from the stools of 3, and from bottled feedings, antiseptic solutions for the hands of nurses, from thermometers and from receptacles in which thermometers were kept. In a 2nd epidemic, *E. coli* was recovered from the throats of 7 patients and from feedings, thermometers and hand dips. A 3rd experience gave cultures of *Staph. aureus* from supposedly sterile nipples, a funnel for filling nursing bottles, the throats of 2 patients, and from the antiseptic solution used for hands. Prepared feedings frequently gave bacterial counts that would not meet requirements for market milk. A variety of bacteria were recovered; from 1 sample, with a bacterial count of 32,000 per cc., *E. coli*, *Staph. aureus*, *B. proteus vulgaris* and Group D streptococci; from another with 396,000 bacteria per cc., *Staph. aureus*, *B. subtilis* and *E. coli*. Even breast feeding may carry a risk, for in 1 outbreak[54] the skin of the breasts of mothers was contaminated by pledgets from a faulty boric acid solution. No sugges-

tion is made that the bacteria recovered under these several circumstances had etiologic relation to the disease. The observations do indicate an exchange of infectious material between patients and their environment. This might well include the actual agent.

Common use of rectal thermometers is prohibited by sanitary regulations, but individual thermometers were not always found available for each infant. Antiseptic solutions for maintaining thermometers were many times ineffective. Experimentally, the addition of small amounts of protein in the form of nutrient broth gave ability to support bacterial growth[78]. As with thermometer solutions, antiseptic solutions for treating the hands between care of individual babies were often at fault. The so-called "hand dips" were often used for several days without change. The bacterial count of one was 150,000 per cc. Contaminated oil bottles and lubricating jellies for thermometers were other possible means of transmitting infection, as judged by bacteriologic studies.

Overcrowding of nurseries was commonly met in investigation of epidemics. A recognized standard[20] for floor space is 30 square feet per bassinet and 40 for premature infants. In 3 epidemics of our experience, there was scarcely room for a nurse to pass between rows of bassinets.

Hypotheses of Origin and Transmission. From information currently available, the need now is to derive a working hypothesis of the origin and mode of transmission of epidemic diarrhea of the newborn, to the practical purpose of permitting as logical a program for prevention and control as is possible. Administrative action must be chiefly on clinical and epidemiologic information. Little or no positive aid is to be had from laboratory procedures, ordinarily a main reliance in infections. The situation is not unlike that which John Snow[76] faced in the control of cholera, except that to modern eyes the problem seems more difficult[30]. The etiologic agent is unknown. Whether or not an acquired immunity results is unknown. There are no known means of inducing artificial immunity. Older infants and adults are presumed to be resistant, but there is no proof that they are. Post mortem examinations show remarkably few tissue changes and none pathognomonic of the disorder.

Origin. The circumstances under which unfamiliar infections appear in a community need understanding for satisfactory appraisal of the present problem. In the first place, a newly recognized condition may be anything but a new disease; it may be among the oldest and best known of infectious processes, strayed from its accustomed habitat to a new location. The invasion of the United States by cholera[19] in earlier times was of this nature.

Occasionally, a primary disease of animals passes successfully to man, to become recognized as a new infection of humans, modern examples being tularemia[61] and rickettsialpox[46].

A number of diseases that came from animals, arthropod-borne encephalitis[65] and Q fever[29], and others such as primary atypical pneumonia[22], give every indication by historical and epidemiological evidence of having existed in human populations long before they were discovered. A new disease may thus be not so much new as newly identified.

Other circumstances give more justification for looking upon an infectious disease as new. The viruses of influenza are notably unstable[13] and proof exists that mutants come into being as new strains within relatively brief periods, epidemiologically considered. Burnet and Clark[16] have advanced that concept in explanation of the pandemic of influenza of 1918.

If the ecologic determination[42] of

mass disease is accepted, if the causes of frequency variations are resident in the qualities of the environment and of the host as well as in the agent, then the origins of new diseases are to be sought also in these other elements of the ecologic triad[41]. Poliomyelitis became a new disease of North American Eskimos[68] by reason of an altered social environment. To the extent that it was recognized as a specific and direct hazard of selected groups, the same infection became a new disease of tonsillectomized persons[7], and by reason of an altered host factor.

Somewhere about 1937, in both Europe and America, appreciation became general that an uncommon acute infection of the intestinal tract was being seen among infants, particularly newborn infants housed in nurseries. The epidemics that occurred were no new problem. Observations had been accumulating for 10 years, perhaps 20, at first as scattered and indefinite episodes, then more frequently, until weight of numbers gave evidence of a significant problem of a peculiar age group. Epidemics welled up over national areas and in several continents. This was no old disease spreading by continuity from a remote endemic source. The behavior was not that of a new microbial variant introduced into fresh populations, with sharp epidemic prevalence affecting all ages. Nothing in its epidemiologic relationships indicated a connection with animals. The slow and spotty increase in numbers of epidemics suggested an origin within populations, from an infectious agent long established there. Either an altered host or a changed environment could have provided the stimulus. No reason exists for a belief that innate host characteristics of infants have been modified during the past 25 years. Significant changes in environment have occurred, particularly in the social environment, whereby in countries chiefly affected most babies are now born in hospitals instead of homes. The aggregation of newborn infants in nurseries provides a physical environment strikingly different from that of the single infant in a home and, presumably, the circumstances which make extensive and highly fatal epidemics of diarrhea possible.

Nature. At least 6 possibilities may be advanced in explanation of the nature of the disease. The 1st is that the syndrome described as epidemic diarrhea of the newborn is a nutritional disorder or a metabolic disturbance with unusually severe consequences because of the time of life at which it occurs, before adjustment to an ordered dietary regimen. This is unlikely because there is no good evidence of differences in attack rates between natural and artificially fed babies. The disease is almost wholly of infants in nurseries; and a nutritional origin would suggest more frequent occurrence in the general population among infants born in homes.

Secondly, there is the possibility that the disease is primarily a toxemia rather than an infection, and arising from a variety of organisms, a feature incident to establishment in the intestinal tract of infants of the normal bacterial flora of humans. The evident communicability is the best evidence against an essential toxemia. Additionally, well-marked differences in intestinal flora characterize natural and artificially fed infants. If the syndrome is related to the initial implantation of foreign microorganisms, then the 2 groups should react differently; and yet both are equally susceptible to epidemic diarrhea and the disease follows a similar course. There is also the matter of restriction to nurseries.

A 3rd assumption is that the condition is not primarily of the intestinal tract but an infection of some other part or anatomic system, as of the

endocrines or the central nervous system, with the intestinal effect of parenteral nature. Clinical manifestations of uncomplicated cases center so strongly around the gastrointestinal tract as to discount a parenteral origin. Other than for pyogenic complications, little tissue change is found elsewhere, but those of the intestinal tract are also far from distinctive. The observations of Buddingh and Dodd[15] may be offered in support of this interpretation, but the outbreak from which they came was not typical of the syndrome under discussion. This explanation is dropped from consideration largely by reason of negative evidence.

The 4th hypothesis now receives consideration. A number of different epidemic intestinal diseases may become implanted in nursery populations. The syndrome described as epidemic diarrhea of the newborn thus may be considered not as a disease entity but as a collection of infections, some caused by known pathogenic agents and some by agents as yet unknown. Children are also subject to several kinds of disease with rash, but this does not discount the validity of measles as an entity. Salmonellosis and shigellosis are 2 specific diseases among an intestinal group. Collectively, they are responsible for a minor component in the total number of recorded epidemics among newborn infants.

Fifth, epidemic diarrhea of the newborn is considered as a specific communicable disease conforming as regularly to type, clinically and epidemiologically, as do most such entities. The clinical and epidemiologic variations shown by the larger group are compatible with a single entity. That assumption is not of necessity required, for 2 or more allied conditions may be represented, of the nature of the typhoid and paratyphoid fevers, to be separated only by more refined methods than now exist. Rather clearly, the majority of outbreaks conform to a pattern which has been described as epidemic diarrhea of the newborn and is reasonably recognized as a specific infectious disease.

The above interpretation does not exclude a final or 6th hypothesis, that the disease represents the reaction of an extremely susceptible host, the newborn infant, to a first impact with an infectious agent, widely distributed in the population and of low virulence for all except infants. If this be true, the ordinary bacterial pathogens appear excluded. To be sure, there are common bacterial diseases of far greater importance for infants than for older children, such as whooping cough, but not to the extent that exists in this instance. Furthermore, the bacterial findings are too irregular. The immediate thought is of a virus. There must be many viruses that conform to the stated characteristics, to consider only the repeated transient fevers of undetermined origin that occur in infancy and childhood and disappear with age. In fact, species are being isolated and identified with increasing frequency, and, like viruses in general, the tendency toward inapparent clinical manifestations is often a predominant characteristic. The undue susceptibility of older children and adults to minor indeterminate fevers as they enter a new environment, characteristically respiratory infections in temperate regions and intestinal in the tropics, is additional evidence of the existence of such viruses. The Goums never did understand the intestinal difficulties of American soldiers in North Africa. At least 4 outbreaks of epidemic diarrhea of the newborn have been related to a virus[55a].

Mode of transmission. Increasing evidence suggests that epidemic diarrhea of the newborn is not so strictly

limited to the neonatal period as was once thought, and that older infants and children develop the infection. An infectious agent so highly selective that it attacks only persons within a specific 4 weeks of age is surely unusual. Ecologically, a parasite with activities thus limited would have difficulty in assuring survival through passage to fresh hosts. The broad geographic distribution of this epidemic disease of infants, in North America[36c], Europe[21,33], Australia[17], Asia[44,57], of itself suggests an agent widely disseminated in human populations. A virus has recently been demonstrated in acute diarrheal disease of adults[69]. The possible relationship needs investigation.

Once introduced into an infant population, the infection persists tenaciously in spite of isolation of patients and quarantine of contacts. This suggests something more than case to case transmission. Inapparent infections of infants and also of adult nursery attendants is one possibility. A carrier state after clinical recovery seems less likely. The virus of Light and Hodes[35a] has been proved decidedly resistant to heat and other environmental influences, which suggests dust and contaminated nursery materials as practical vehicles of infection. Experimental transmission of diarrhea of adults to human volunteers[40] has been accomplished not only by stool suspensions, but by respiratory secretions. In the absence of direct knowledge, control measures for epidemic diarrhea of the newborn should be sufficiently broad to include both routes.

Working hypothesis. A practical program of control is believed advisedly based on a working hypothesis of origin and transmission that includes: 1. Epidemic diarrhea of the newborn is a specific communicable disease, presumably caused by a virus or group of related viruses of high infectivity for newborn infants; the reservoir of infection is the human host; the infection has been long implanted in American populations; the newly observed epidemiologic behavior as an epidemic disease of the newborn is primarily determined by changes in the social environment, whereby most births are in hospitals and infants are aggregated in nurseries; 2. Infants beyond the neonatal period may develop the clinical disease; 3. Once introduced into nurseries, infection tends to persist obstinately and for long periods; the reasons are unknown. Control measures should give attention to inapparent infection of infants and of adult nursery attendants, to a carrier state among recovered patients, and to the ability of the virus to survive for appreciable periods outside the human body; 4. Knowledge of the natural mode of spread is indefinite. Since a similar disease of adults has been transmitted experimentally by materials from both the upper respiratory and intestinal tracts, control measures are to be directed toward both modes of spread. The recommendations for prevention and control that now follow are on these assumptions.

Control of Epidemics. Outbreaks of acute diarrhea of the newborn are a problem of hospital nurseries. No epidemic has been recorded in a general population. Recognition that an epidemic exists or is in the making is a basic consideration. A specifically designated hospital staff member should be consulted in all suspicious cases. Each nursery attendant has the obligation to know and to report the disease. The recent manual, *Control of Communicable Diseases in Man*[6], by the American Public Health Association, is a source of information and of what to do in prevention and control. so inexpensive as to be universally available. Without equivocation, sus-

picious cases are to be segregated from well babies until diagnosis is established. Early report of outbreaks to the official health agency is fundamental to control. These events have wider significance than the local hospital interest. The health department coordinates the activities of hospital officials, medical staff, local health authorities and the public.

Once an outbreak is established, the nursery is closed to admissions. Sick infants are placed in isolation in the pediatric ward or in another hospital. In some instances[80] admission of newborn infants to a separate clean nursery has continued during an outbreak. In our experience, a clean nursery has even been operated adjacent to the contaminated unit without cross infection. The practice involves a risk, for despite strict attention to aseptic technique, cross infection has occurred[72].

Contacts are quarantined and observed for early signs and symptoms of the disease. Through arrangement with public health officers, exposed healthy infants discharged to their homes during the outbreak are followed for evidence of subsequent illness, mothers being informed of the early manifestations and of the necessity for prompt treatment. Search for cases among admissions to other hospitals has revealed missed cases and even unreported outbreaks. Death certificates of newborn infants during the epidemic period may also give information.

Had the practice not been a matter of observation, it would be superfluous to advise that non-professional attendants are not to serve both clean and infected nurseries. Infants should not be taken from contaminated nurseries to be fed by mothers. The entire maternity unit, including nursery, labor and delivery rooms, and the formula preparation room are scrutinized for faults in aseptic technique and inadequacies of equipment. Sanitary inspection is made of water and milk supplies and of food storage. The obstetrical and nursery departments should be thoroughly cleaned after final discharge of patients and exposed infants, equipment sterilized where possible, and sanitary defects corrected.

Prevention of the Disease. Prevention of epidemic diarrhea of the newborn requires analysis of medical and nursing supervision, of physical construction of hospital facilities, and of medical and surgical techniques. For better appraisal of maternity units, a scoring system has been devised, with numerical values assigned each of 8 factors regarded as significant[73].

Immediate control of the nurseries is desirably assigned to 1 physician, an experienced pediatrician. Nursing supervision is likewise specifically delegated. New nurses require special instruction before assignment to the service, and duties are limited to the nursery, with necessary relief recruited from parts of the hospital not concerned with infections.

Labor rooms are recommended in the ratio of 1 labor to 10 maternity beds, and 1 delivery room for each 20 beds, except that in large specialized maternity hospitals lesser requirements suffice. Adequate provision is required for maternity patients with infections. Gynecologic cases are preferably not housed on the maternity floor, and segregation from medical or surgical cases is desirable. In smaller hospitals, housing of the entire maternity unit on a single floor will decrease infant travel through corridors and on the elevator. Elevators used by nurses to transport infants should be free of other passengers, but provided with an operator.

Planning of new nurseries and the remodelling of existing facilities re-

quire attention to possible avenues of infection. Adequate space, light, ventilation and controlled humidity should be considered. Suspension of bassinets on double-decked pipe racks is prohibited. A common practice of the past was to build large nurseries with 20 to 30 bassinets. Present day recommendations are for smaller units, desirably to house 12 full-term infants, and ideally 8 to 10 bassinets, although such small units introduce difficulties in adequate utilization of nursing time.

Massachusetts hospital regulations[58a] require nurseries to be provided with an anteroom to serve as an examining room for physicians. This design provides a series of units, with the result that contamination of one does not interfere with activities in another, as in conventional construction with a single workroom for each 2 nurseries. A unit with the specific purpose of housing suspicious cases is another aid in control of infectious diarrhea of the newborn. Transfer of infants to isolation was formerly much delayed, often until the diagnosis was definite, with the result that all infants in the nursery were exposed. Infants with suspicious symptoms are transferred for observation at the discretion of the supervisor or head nurse, are cared for there by attendants with no duties in the nursery, and cannot be removed until symptoms are accounted for. This special unit, with its anteroom, is wholly separate from the regular nursery and provides 2 and not more than 3 bassinets. For the small hospital, one such bassinet is recommended for each 10 of capacity, a ratio in excess of requirements for large maternity hospitals. Isolation facilities are in addition, for the suspect nursery is not used for recognized infections.

The recommended practice for infant care is that it be in the infant's bassinet, with individual equipment properly labelled and kept in a built-in storage space for each bassinet. Such unit care insures a degree of isolation. Infants are preferably transported to mothers at feeding time in the arms of the nurse or in individual bassinets, and not by group carriers. Experience in the investigation of epidemics shows that formula preparation in hospitals[61] does not always receive merited attention, notably the periodic review[71] of sterilization procedures, refrigeration and the sanitary environment in which foods are made. Common dressing or diaper tables are undesirable. They foster cross infection. Weighing scales are draped with sterile material before weighing babies. Handwashing by attendants with soap or a detergent is important. Dipping the hands in presumed antiseptic solution is not adequate. Individual lavatories are a part of each nursery.

Visiting of maternity units by persons with colds, sore throats and diarrheal disease is not to be permitted. In the face of an outbreak, all visiting enlarges the possibility of spread of infection, and by choice is suspended. The practical solution is most often found in limiting the number of visitors and of visits.

The current resumption of the rooming-in principle[10,47] brings new problems of prevention, not only of epidemic diarrhea of the newborn but of other infections of the neonatal period. Where service is provided through private rooms with few attendants, there is no problem. Indeed, this may be the answer to the epidemics of recent years, for the environmental effect is assuredly not of hospitals as such, but of nurseries, of aggregation of infants and the resultant opportunity for spread of infection. On the other hand when rooming-in is accomplished through 4- and 8-bed units, with two 4-bed bassinet nurseries and a common workroom, the smaller units are a gain but the number of contacts be-

tween mothers, infants, nurses and visitors is greatly increased.

Summary. The empirical measures that are the current program for prevention of epidemic diarrhea of the newborn exist for a single reason, and that is lack of knowledge. What is done in the face of an epidemic recalls a cherished army expression of "throw the book at it"—try everything. Outbreaks are controlled, but with little more finesse than the veterinarian has when he meets his epidemic problems by the test and slaughter method.

What needs most to be known, other than the identity of the infectious agent, is the source of infection. Information might well be sought in the relationships between epidemics of the newborn, the outbreaks that occur so frequently in schools, and those of older adults in institutions. In each class, some are demonstrably of orthodox origin, by way of the common intestinal bacteria; others are of obscure etiology.

Epidemiologic interests experience fashions, as does most human endeavor. The early concern with intestinal infections and the problems of environmental sanitation later turned to respiratory disease and then to the infections transmitted by arthropods. Those who feel that the first field has been largely exhausted, that environmental sanitation is no longer a consequential part of public health, might have a look at the nurseries of present day hospitals.

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BOOK REVIEWS AND NOTICES

ANTIHISTAMINIC AGENTS IN ALLERGY. By 18 Authors. Annals of the New York Academy of Sciences, Vol. 50, Art. 9, Pp. 1013-1208. New York: The Academy of Sciences, 1950. Price, $3.00.

FOURTEEN articles that discuss the various aspects of the role of histamine in human physiology and in experimental anaphylaxis; the detoxification of histamine; antihistaminic agents: their mode of action under various conditions and their application in clinical allergy. The Authors are experts in their several fields (*e.g.*, The Pharmacology of Histamine, by Sir Henry Dale; Antihistamine Therapy, by Samuel M. Feinberg). This symposium should therefore be welcomed by those interested in experimental and clinical allergy. R. K.

CYTOLOGIC DIAGNOSIS OF LUNG CANCER. By SEYMOUR M. FARBER, M.D., MILTON ROSENTHAL, M.D., EDWIN F. ALSTON, M.D., MORTIMORE A. BENIOFF, M.D., and ALLEN K. MCGRATH, JR., M.D., Univ. of California. Pp. 59; 60 ills. in color. Springfield, Ill.: Charles C Thomas, 1950. Price, $6.00.

THIS monograph details the problem of recognizing cancer cells in sputum and bronchial aspirates, ranging from collection and preparation of material to differentiation from normal cells. The Authors emphasize reliability (with a virtual absence of "false positives") at the expense of sensitivity, reporting positive cytologic diagnoses in 55% of "proven" bronchogenic carcinomas and in 64% of cases in which diagnosis was made by bronchoscopic biopsy. Some pathologists may not agree with the list of advantages of the cytologic method over bronchoscopic biopsy. The photomicrographs, excellent and all in color, would be more valuable if they were inserted in the text at appropriate points and if more of the magnifications were uniform.
R. H., Jr.

FUNDAMENTALS OF PHYSICAL CHEMISTRY FOR PREMEDICAL STUDENTS. By H. D. CROCKFORD, Prof. of Chemistry, and SAMUEL B. KNIGHT, Prof. of Chemistry, Univ. of North Carolina. Pp. 366. New York: John Wiley & Sons, 1950. Price, $4.25.

THE text presents clearly and simply many of the fundamental aspects of physical chemistry. Among the topics considered are the various states of matter, osmotic pressure, electrolytes, buffer action, electromotive force, pH determinations, colloids, and radioactivity. There are numerous illustrations and many thought-provoking problems with answers. Practically all references are to other books, rather than to recent research papers. The clarity of presentation and the absence of complicated mathematics help make this book favorable for students not specializing in the field. Otherwise, it adds little to what is already present in other texts of the same type. I. R. Z.

A PRIMER OF VENOUS PRESSURE. By GEORGE E. BURCH, M.D., Prof. of Medicine, Tulane Univ. Pp. 174; 170 ills. Phila.: Lea & Febiger, 1950. Price, $4.00.

THE Author of this new book states that it "is intended for the beginner . . . designed to arouse his interest . . . so that he might better understand and manage his patient. Certain fundamental principles concerned with the maintenance of and fluctuations in venous pressure are discussed both in normal and disease states to illustrate how adequate appreciation of their significance may permit more intelligent analysis of clinical syndromes."

Under the chapter headings of functional anatomy, physiology and hemodynamic phenomena, measurements of venous pressure, normal values, and values in disease states, the Author presents the fundamentals in a clear and easily readable style. At least half of the space is occupied by illustrations, mostly line drawings. Many of these present material in so over-simplified a manner that, much as the Reviewer likes adequate illustration, this seems to be overdoing it a bit. Certainly the anatomical section would have been improved by the use of actual photographs or photomicrographs.

The purpose or usefulness of a "primer" devoted to venous pressure is not quite clear. Such a specialized topic would seem to be a proper subject for a monograph, not an elementary study. The book seems to be aimed at medical practitioners, but the Reviewer doubts that it will appeal to them. It might have usefulness for medical students, but the over-simplification, lack of discussion of controversial points and lack of a bibliography would greatly limit its use even here. W. S.

THE 1949 YEAR BOOK OF PEDIATRICS. Edited by HENRY G. PONCHER, M.D., with JULIUS B. RICHMOND, M.D. Pp. 560; 120 ills. Chicago: Year Book Publishers, 1950. Price, $4.50.

THIS survey of pediatric progress in 1949, under the able direction of the new editors, is excellent. Many pertinent editorial comments are interspersed. The text covers the period from July 1948 to July 1949. I. W.

HUMAN FERTILITY AND PROBLEMS OF THE MALE. By EDMOND J. FARRIS, Ph.D., The Wistar Institute of Anatomy and Biology. Pp. 211, 33 figs., 5 color plates. White Plains, N. Y.: The Authors Press, 1950. Price, $5.00.

IN this book, the Author presents part of the results of his investigations of human reproduction. He deals chiefly with the problems of the male, but includes observations on ovulation and certain practical aspects of barren marriage. The material is fresh and brings into proper focus the known factors in male fertility. There are sections, for example the evaluation of treatment of male infertility, wherein the data are not sufficient. This is recognized by the Author, and detracts but little from the value of the book. Physicians and others interested in reproduction will find the book stimulating and practically helpful. P. K.

NEW BOOKS

Some Relations between Vision and Audition. By J. DONALD HARRIS, Ph.D., U. S. Naval Med. Res. Lab., New London, Conn. Am. Lect. Ser. 71. Pp. 56. Springfield, Ill.: Charles C Thomas, 1950. Price, $1.50.

A Study of Diphtheria in Two Areas of Great Britain. By PERCIVAL HARTLEY, *et al.* Pp. 162. Med. Res. Council, Special Rep. Ser. 272. London: H. M. Stationery Office, 1950. Price, 4s.

The Cerebral Circulation in Health and Disease. By CARL F. SCHMIDT, M.D., Lab. of Pharmacol., Univ. of Penna. Am. Lect. Ser. 68. Pp. 78. Springfield, Ill.: Charles C Thomas, 1950. Price, $2.00.

Biochemical Aspects of Genetics. Edited by R. T. WILLIAMS. Symposia No. 4, Westminster Hosp. Med. School, London. Pp. 60. New York: Cambridge Univ. Press, 1950. Price, $1.50.

The Hormones, Physiology, Chemistry and Applications. Vol. 2. Edited by GREGORY PINCUS and KENNETH V. THIMANN. Pp. 782. New York: Academic Press, 1950. Price, $12.50.

THIS is the second of a 2-volume compilation prepared by 24 authoritative contributors. The first volume was reviewed in this journal in August, 1949. The present volume contains chapters on the physiology of ovarian hormones, androgens, the thyroid and its hormones, the adrenal cortex, and the gonadotrophins; the hypophysis and diabetes mellitus; the hormones of the posterior pituitary; the chemical control of nervous activity; and clinical endocrinology. With due allowance for the inevitable lag of publication behind the rapid march of endocrinology, these volumes wil serve as a useful source of reference in the field of endocrine physiology and biochemistry. E. R.

Centennial. Collected Papers. Preface by EDMUND W. SINNOTT, Yale Univ. Pp. 313; illustrated. Washington, D. C.: American Association for the Advancement of Science, 1950. Price, $5.00.

THE centennial meeting of the A.A.A.S. was celebrated by a series of symposia and lectures given by distinguished invited guests. Of the 13 groups into which they have been classified in the present volume, all but 1 or 2 extend into the fields of medical sciences, and in 2 or 3, such as Nutrition, World Health, Human Individuality, Sound Waves and Rhythms, the contributions are mainly in these fields. The reliability of the presentations will be taken for granted; what single individual could hope to do more? E. K.

Primary Carcinoma of the Vagina. By ROBERT G. LIVINGSTONE, M.D., Memorial Hosp. for Treatment of Cancer and Allied Diseases, New York. Am. Lect. Ser. 38. Pp. 73. Springfield, Ill.: Charles C Thomas, 1950. Price, $2.00.

Maternity Care in Two Counties, Gibson, Tennessee, and Pike, Mississippi, 1940-41, 1943-44. By FRANK E. WHITACRE, M.D., and ELLEN WHITEMAN JONES, M.P.H. Pp. 165. New York: Commonwealth Fund, 1950. Price, 50 cents.

Kompendium der Homöopathischen Therapie. Von Dr. med. JULIUS MEZGER. Pp. 151. Stuttgart, Germany: Marquardt & Cie., 1950. Price, D.M. 8.75.

Clinical Uses of Intravenous Procaine. By DAVID J. GRAUBARD, M.D., Kingston Avenue Hosp., Brooklyn, N. Y., and MILTON C. PETERSON, M.D., Research Hosp., Kansas City, Mo. Am. Lect. Ser. 73. Pp. 104; 8 ills. Springfield, Ill.: Charles C Thomas, 1950. Price, $2.25.